Supporting Information

Yoshida et al. 10.1073/pnas.0907896106

SI Text

SANG SANG

Cover Caption. The spikelet of rice (*O. sativa*) forms a unique organ, sterile lemma, between a floret and two rudimentary glumes. A mutation in G1 causes homeotic transformation of the sterile lemma into the lemma. G1 encodes a land plant-specific

protein containing an uncharacterized domain, named ALOG. Yoshida et al. suggest that *G1* may have been involved in morphological modification of the spikelet during the evolution of rice.



Fig. S1. Spikelet development in WT (*A*–*I*) and *g1* (*J*–*R*). (*A*–*C*, *J*–*L*) Developmental patterns of spikelets in WT and *g1*. Each organ is indicated by color: blue, floral meristem; orange stamen; yellow, palea; green lemma; pink, sterile lemma in WT and LSL in *g1*. (*D*–*F*, *M*–*O*) Epidermal surface of the lemma in WT (*D*–*F*) and *g1* (*M*–*O*). (*G*–*I*) Epidermal surface of the sterile lemma in WT. (*P*–*R*) Epidermal surface of the LSL in *g1*. (Scale bars: 100 µm in *A*–C and *J*–*L*; 10 µm in *D*–*I* and *M*–*R*.)

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Fig. S2. Phenotypes of spikelets in *g1* mutants and *O. grandiglumis*. (*A–D*) Spikelet phenotypes of *g1–1*, *g1–2*, *g1–3*, and *g1–4*. (*E*) RT-PCR analysis of *G1* mRNA in young inflorescence. Among the three genes in the 12-kb deletion (Fig. 2*A*), only Os07g04670 is expressed in developing spikelets. (*F–H*) Cross-section of the spikelets of *O. grandiglumis*. A spikelet (*F*) and close-up views of marginal region of the lemma/plea (*G*) and LSL (*H*) are shown. (Scale bars: 1 mm in *A–D*; 500 μ m in *F*; 100 μ m in *G* and *H*.)

AGACGCATTCTGTCACGTCTCTCTAGTGTTACTGCGTAACGTAGGTGGCCGCTGCCGGCC

ATGTCGTCGTCGTCGCTGCCGCGCGGGGCTGGCGGGGGGGG	G 20
CGGCCGAGCCGGTACGAGTCGCAGAAGCGCCGGGACTGGCAGACCTTCACGCAGTACCT R P S R Y E S Q K R R D W Q T F T Q Y L	C 40
GCCGCGCACCGCCGCCGCTCGAGCTCCGCCGCGCGCGCCCACGTCCTCGAGTT	C 60
CTCCGCTACCTCGACCGCTTCGGCAAGACGCGCGCGTCCACGAGCCGCCGTGCCCGTCGTA L R Y L D R F G K T R V H E P P C P S Y	C 80
GGCGGCCGCCGCCGCCGCCGCCGCCGCCGCCGCCGCCGC	C 100
CCGCTGCGCCAGGCGTGGGGGGGGGGGCAGCCTCGACGCGCCTCGCGCCGCCTA PLRQAWGSLDALVGRLRAAY	C 120
GACGAGCGTCACGGCCGCGCCGCCGCGGGGGGGGCGCCGGGGGGGG	(<i>g1-3</i>) C 140
ACCGACAGTACCTCCTCCTCCGCCGCCGCCGCCGCCGCCCCGCGCGCG	G 160
AGGCTGTACCTGCGCGACGTCCGCGACGCGCGGCATGGCGCGCGC	C 180
AAGAAGAAGAAGCGCAGGGGGGGGGGGGGGGGGGGGGG	<u>C</u> 200
GCGCGCGCGGGGCGTGAACGACGGCGGCGGCGGCGGCGGCGGGGGGGG	<u>G</u> 220
CTACCTCTGCCGCCGCTGCCACCGTGCCTCAACGGTGTGCCGTTCGAGTACTGCGACTT L P L P P L P P C L N G V P F E Y C D F	<u>C</u> 240
GGGAGCGTCCTCGGGGGAGCACATGGCGCCCATGGCGGCCATGGCGGCGGCGGCGGCGGCGGCGGCGGCGGCGGCGGCGGC	<u>C</u> 260
TTCTACGGCGCCGGCGTCTACTTGCCATTTCTGTACAACACCTTCAGTTAGTT	<u>C</u> 276

TAGCTAGTTCGTCGTGTATTTGTCTGTGCTTCTCACTGTGGTTGCTTCAGTGTACTAGCT

<u>AGCTACATGTGTGATGTGTCTGCATCTTGTG</u>ATCATCTTGATCTGTGCTTTGATGCTGAT

Fig. 53. DNA and protein sequences of rice G1. Blue and red boxes indicate the ALOG domain and nuclear localization signal, respectively. Thick lines indicate amino acid insertions in the ALOG domain. Underline indicates the region used for in situ probe. Mutations in the g1 alleles are shown in orange.

SANG SANG

0-01	1		0.0
USGI	1	SRTESQRRDWQTFTQTLAAHRPPLELRRCSGAHVLEFLRTLDRFGRTRVHEPPCPSTGGRSPSAAGPVAAAAAAACQCPL	80
ZmG1	1	SRYESQKRRDWQTFTRYLGAHRPPLELCRCSGAHVLEFLRYLDRFGKTRVHAPSCAAYGGGGGGGGGRPVEAAVACQCPL	77
SbG1	1	SRYESQKRRDWHTFTRYLAAHRPPLELCRCSGAHVLEFLRYLDRFGKTRVHAPLCAAYGGGGGGPALVAAAPGQCPL	77
BdG1	1	SRYESQKRRDWATFTRYLAAHRPPLELRRCSGAHVLEFLRHLDRFGKTRVHIPHCPSYGGAALSSSTANT-HFHPQQCPL	79
OsG1L1	1	SRYESQKRRDWQTFGQYLRNHRPPLELSRCSGAHVLEFLRYLDQFGKTKVHAHGCPFFGHPSPPAPCPCPL	71
0sG1L2	1	SRYESOKRROWHTFGOYLRNHRPPLELSRCSGAHVLEFLRYLDOFGKTKVHAAGCPFFGHPSPPAPGPCPL	71
0sG1L3	1	SRYFAOKRROWNTEGOYLRNHRPPL GLAOCSGAHVLEELRYLDOEGKTKVHTAACPEEGHPNPPAPCPCPL	71
056114	1		71
066115	1		71
050115	1		71
USGILO	T		71
USGIL/	1	SRYESQKRRDWNTFLOYERNHRPPLTEARCSGAHVIEFLRYLDQFGKTKVHASGCAFYGQPSPPGPCPCPL	/1
OsG1L8	1	SRYESQKRRDWNTFLQYLKNHRPPLTLARCSGAHVIEFLKYLDQFGKTKVHASGCAYYGQPSPPAPCPCPL	71
OsG1L9	1	SRYESQKRRDWNTFLQYLRNHKPPLTLPRCSGAHVIEFLKYLDQFGKTKVHADGCAYFGEPNPPAPCACPL	71
AtLHS1	1	SRYENQKRRDWNTFCQYLRNHRPPLSLPSCSGAHVLEFLRYLDQFGKTKVHHQNCAFFGLPNPPAPGPCPL	71
AtLHS2	1	SRYENQKRRDWNTFC0YLRNHHPPLSLASCSGAHVLDFLRYLDQFGKTKVHHQNCAFFGLPNPPAPCPCPL	71
AtLHS3	1	SRYENOKRROWNTFGOYLRNHRPPLSLSRCSGAHVLEFLRYLDOFGKTKVHTNICHFYGHPNPPAPGPCPL	71
AtLHS4	1	SRYENOKRRDWNTEGOYLRNHRPPLSLSRCSGAHVLEELRYLDOEGKTKVHTHLCPFEGHPNPPAPCACPL	71
A+1 HS5	1		71
A+1 HS6	1		71
	1		71
ALLHS7	1		71
ATLHS8	1	SKTESQKSRDWNTFCQTCMTKMPPVHWECESNHILDFLQSRDQFGKTKVHIQGCVFFGQKEPGECNCPL	/1
AtLHS9	1	SRYESQKRRDWNTFVQYLKSQNPPLMMSQFDYTHVLSFLRYLDQFGKTKVHHQACVFFGQPDPPGPCTCPL	/1
AtLHS10	1	SRYESQKRRDWNTHGQYLKNQRPPVPMSHCSCNHVLDFLRYLDQFGKTKVHVPGCMFYGQPEPPAPCTCPL	71
Sm G1-like1	1	SRYEAQKRRDWNTFGQYLKNHRPPLALSRCSGAHVLEFLRYLDQFGKTKIHAPACPFFGLAHPPAPCACPL	71
Pp G1-like1	1	SRYEAQKRRDWNTFGQYLRNHRPPLALARCTGVHVLEFVHYLDQFGKTKVHVPSCPFFGLPHPPHPCPCPL	71
OsG1	81	ROAWGSLDALVGRLRAAYDERHGRAGEPDAVAGAGAVATDSTSSSSA-AAANPFAARAVRLYLRDVRDAQAMARGISY	157
	70		152
SDGI	10		100
Bagi	80	QQAWQSLDALVGRLRAAFADNDNDNNKASINPFAARAVRLYLRDVRDTQSKARGISY	136
OSG1L1	72	RQAWGSLDALVGRLRAAFEEHGGR	125
OSG1L2	72	RQAWGSLDALVGRLRAAFEEHGGRPEANPFGARAVRLYEREVRDSQAKARGIAY	125
OsG1L3	72	RQAWGSLDALV <mark>GRLRAA</mark> FEENGGRPESNPFAVRAVRLYLREVREHQARARGVSY	125
OsG1L4	72	RQAWGSLDALVGRLRAAFEENGGRPESNPFAARAVRLYLREVREHQARARGVSY	125
OsG1L5	72	RQAWGSLDALVGRLRAAYEENGGRPENNPFGARAVRLYLREVREHQARARGVSY	125
OsG1L6	72	RQAWGSLDALIGRLRAAYEENGGTPEMNPFGARAVRLYLREVRETQARARGISY	125
OsG1L7	72	RQAWGSLDALIGRLRAAYEESGGTPESNPFAARAVRIYLREVRDSQAKARGIPY	125
OsG1L8	72	RQAWGSLDALIGRLRAAYEESGHAPESNPFAARAVRIYLREVRDAQAKARGIPY	125
OsG1L9	72	ROAWGSLDALIGRLRAAYEESGGRPESNPFAARAVRIYLREVREAOAKARGIPY	125
AtLHS1	72	ROAWGSLDALIGRLRAAYEENGGPPEANPEGSRAVRLFLREVRDEOAKARGVSY	125
A+1 HS2	72	ROAWGSL DAL TGRERAAY FENGGAPETSPEGSRSVRTEL REVROEOAK SRGVSY	125
A+1 HS3	72	ROAWGSLDALTGRI RAAFFENGGK	125
A+1 HSA	72		125
A+1 HS5	72		126
	72		125
	72		125
ATLHS/	72		125
ATLHSO	72		1 / 5
ATT HVU	72		125
ALLIISS	72 72	KOAMOSLDALIGKLKAAVEENOGLIEKNPFAKGGIRIFIKEVKGSOAKARGVLV KOAMOSLDALIGRLKAAVEEHGGGSPDTNPFANGSIRVHLREVRESOAKARGIPV	125
AtLHS10	72 72 72	KOAWGSLDALIGKLKAAYEENGGLPDTNPFAKGGIRIFLKEVKGSQAKARGVLV KQAWGSLDALIGRLKAAYEEHGGGSPDTNPFANGSIRVHLREVRESQAKARGIPY RQAWGSLDALIGRLKAAYEENGGPPETNPFASGAIRVYLREVRECQAKARGIPY	125 126 125
AtLHS10 Sm G1-like1	72 72 72 72	KUAMUSLUALIGKLKAAFEENGGL	125 126 125 125

Fig. S4. Amino acid alignment in the ALOG domain. Thick red lines indicate the insertions found in protein in the G1 clade.

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0. sativa	MSSSSAAALG	SDDGCSPAEL	RPSRYESQKR	RDWQTFTQYL	AAHRPPLELR	50
W0613-1	MSSSSAAALG	SDDSCSPAEL	RPSRYESQKR	RDWQTFTQYL	AAHRPPLELR	
W0613-2	MSSSSAAALG	SDDSCSPAEP	RPSRYESQKR	RDWQTFTQYL	AAHRPPLELR	
W1194-1	MSSSSAAALG	SDDSCSPAEL	RPSRYESQKR	RDWQTFTQYL	AAHRPPLELR	
W1194-2	MSSSSAAALG	<mark>SDD</mark> S <mark>CSPAE</mark> P	RPSRYESQKR	RDWQTFTQYL	AAHRPPLELR	
	* *	* ****	* *	*	* *	
	RCSGAHVLEF	LRYLDRFGKT	RVHEPPCPSY	GGRSPSAAGP	VAAAAAACQC	100
	RCSGAHVLEF	LRYLDRFGKT	RVHESPCPAY	GGRALSAAGP	VV <mark>AACQC</mark>	
	ALQRRPRPRV	PPLSRPLRQD	ARPRVAVPGI	RRPRAVRRRP	GRRRLPVPAA	
	RCSGAHVLEF	LRYLDRFGKT	RVHESPCPAY	GGRALSAAGP	VV <mark>AACQC</mark>	
	RCSGAHVLEF	LRYLDRFGKT	RVHESPCPAY	GGRALSA PPR	PASPPASARC	
	** ******	** *****				
	PLRQAWGSLD	ALVGRLRAAY	DERHGRAGEP	DAVAGAGAVA	TDSTSSSSAA	150
	PLRQAWGSLD	ALVGRLRAAY	DERQGRAGEP	DAAA-AGAVA	TDSTSSSSAA	
	PGLG					
	PLRQAWGSLD	ALVGRLRAAY	DERQGRAGEP	DA <mark>AA</mark> -AGAVA	TDSTSSSSAA	
	ARLGVASTRS	SAASAPPTTS	VTAAPGNPTP	PPPALSPPAL	SPPTVPPPP	
	**	* * *	* ** *			
	AANPFAARAV	RLYLRDVRDA	QAMARGISYH	KKKKRRGGNM	NGARGGGGGG	200
	AANPFAARAV	GLYLRDVRDA	QAMARGISYH	KKKKRRGGNM	NGARGGGGGG	
	AANPFAARAV	GLYLRDVRDA	QAMARGISYH	KKKKRRGGNM	NGARGGGGGG	
	PPPPTPSPRA	Р				
	ARAGVNDGDA	TAPPVAVTPG	LPLPPLPPCL	NGVPFEYCDF	GSVLGGAHGA	250
	ARAGVNDGDA	TAPPVAVTPG	LPLPPLPPCL	NGVPFEYCDF	GSVLGGAHGA	
	ARAGVNDGDA	TAPPVAVTPG	LPLPPLPPCL	NGVPFEYCDF	GSVLGGAHGA	
						270
	HGGHGGGGGG	FYGAGVYLPF				276
	HGGHGGGGGG	FYGAGVYLPF	LYNIFS			
	HGGHGGGGGG	FYGAGVYLPF	LYNTFS			

Fig. S5. Amino acid alignment of G1 proteins from *O. sativa* and *O. grandiglumis*. Thick blue lines indicate the ALOG domain and black lines indicate the insertions in G1 proteins. The two genomes of the tetraploid species *O. grandiglumis* (accessions, W0613 and W1194) are tentatively numbered 1 and 2.



Fig. S6. Subcellular localization of G1. (A) GFP-G1 fusion protein. (B) DAPI staining of the nucleus.

Table S1. Accession numbers

Species	Accession	Gene	Accession no.
O. sativa	_	G1	AB512480
	_	G1L1	AB512490
	_	G1L2	AB512491
	_	G1L3	AB512492
	—	G1L4	AB512493
	—	G1L5	AB512494
	_	G1L6	AB512495
	—	G1L7	AB512496
	—	G1L8	AB512497
	_	G1L9	AB512498
O. grandiglumis	W0613	OgG1-1	AB512486
	W0613	OgG1-2	AB512487
	W1194	OgG1-1	AB512488
	W1194	OgG1-2	AB512489
B. distachyon	—	BdG1	AB512481
Z. mays	—	ZmG1	AB512482
S. bicolor	—	SbG1	AB512483
P. patens	—	PpG1-like1	AB512484
S. moellendorffii	_	SmG1-like1	AB512485

Table S2. Primers used in this study

PNAS PNAS

	Primer	Marker	
Mapping			
g1M01 d1	5'-TATCTACGTCAAAGGCAGTC-3'	CAPS (Afal)	
g1M01 u2	5'-TTGAATGTTCACGACATGCA-3'		
g1M02 d1	5'-CATTCATTTATTTTGTACAGATCAC-3'	STS	
g1M02 u2	5'-GCATGCTCCCGGTGTCATTTC-3'		
g1M05 d1	5'-CCTGAAACAAAATGGATGATAA-3'	CAPS (Dral)	
g1M05 u2	5'-ATGTGAATGAAAAACCTAATCC-3'		
g1M11 d1	5'-CCTGAAACAAAATGGATGATAA-3'	CAPS (Pstl)	
g1M11 u2	5'-ATTTATTTATTATGACTTGATTTGT-3'		
g1M19 d1	5'-TGGAACAAGCCGACTGTCATACAC-3'	dCAPS (Ndel)	
g1M19 u2	5'-GAGGTTCAATTTTCTTTTTCTTTTCAT-3'		
g1M28 d1	5'-TGTCACATTAAATATACGGACGTA-3'	dCAPS (Afal))	
g1M28 u2	5'-GATGGATTAATTAGGCTGGC-3'		
g1M32 d1	5'-ATCGTTACGATCGGAAATTTTAA-3'	STS	
g1M32 u2	5'-TGATGGTTTAGTAGCGTTTGTAGA-3'		
G1 cDNA amplification			
G1 d1	5'-ACTACCACCTTGGTTGGCTCTATTAAAGGC-3'		
G1 u2	5'-CACAAGATGCAGACACATCACACATGTAGC-3'		
In situ probe			
G1i d1	5'-CCGGGGAGCCCGACGCCGTCGCGGGCGCCG-3'		
G1i u2	5'-CACAAGATGCAGACACATCACACATGTAGC-3'		
Construction of G1-GFP fusion protein			
G1 gfp d1	5'-G <u>GAATTC</u> ATGTCGTCGTCGTCCGCTGCCG-3'	EcoRI*	
G1 gfp u2	5′-G <u>GAATTC</u> ACTGAAGGTGTTGTACAGAAAT-3′	EcoRI*	
Construction of G1-GAL4BD fusion protein			
G1 gal d1	5'-TCC <u>GATATC</u> GATGTCGTCGTCGTCGCCGCGCGCGGGC-3'	EcoRV*	
G1 gal u2	5'-ACGC <u>GTCGAC</u> CTAACTGAAGGTGTTGTACAGAAATGGCAAG-3'	Sall*	

*Restriction enzyme used for plasmid construction.