

**A**

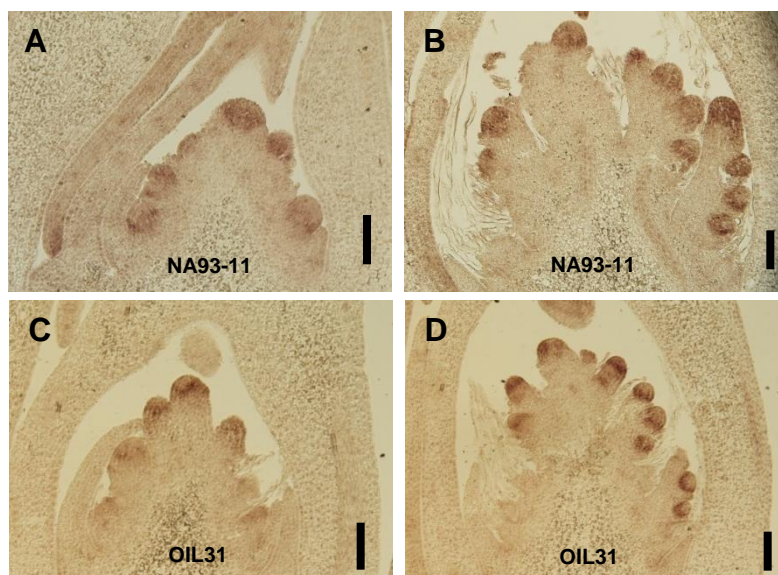
			<b>Indel1</b>
NA93-11	1	ATGAGGACGGCGGCCACGCCGCTCT...CGCCGCCGCCGCCGCCGCCGTCGCGGCAGTG	
OIL31	1	ATGAGGACGGCGGCCACGCCGCTCTCGCCGCCGCCGCCGCCGCCGTCGCGGCAGTG	
			<b>Indel2</b>
NA93-11	58	TTCCTCTCTGCGTTGCTGCTCGCTCCGCTCCGCTCCAGGCTCCCTCCTCCT	
OIL31	61	TTCCTCTCTGCGTTGCTGCT.....CGCTCCGCTCCGCTCCAGGCTCCCTCCTCCT	
NA93-11	118	CGCCGTCTTCTTCCCCTGGTACGCGTACGCCCTCGCCGCCGCCGTTCTTGATCGACCTGA	
OIL31	115	CGCCGTCTTCTTCCCCTGGTACGCGTACGCCCTCGCCGCCGCCGTTCTTGATCGACCTGA	
NA93-11	178	GTGTTTTTTAAAAATTTGTTTCGGTTAGTTGATGCGCTGTGTGTTTCGTCCATGGCGATGC	
OIL31	175	GTGTTTTTTAAAAATTTGTTTCGGTTAGTTGATGCGCTGTGTGTTTCGTCCATGGCGATGC	
			<b>SNP1</b>
NA93-11	238	CGATGCAGGTTGGTGGCGAGGTGGCGGTGGCGGTGGTGGCTGGGAGGAGGAGAAGGTGC	
OIL31	235	CGATGCAGGTTGGTGGCGAGGTGGCGGTGGCGGTGGTGGCTGGGAGGAGGAGAAGGTGC	
NA93-11	298	GGCTGGGGTCGAGCCCGGAGCTGCTACAGCAAGTGCTACGGTGCAGCCCGTGCCTCG	
OIL31	295	GGCTGGGGTCGAGCCCGGAGCTGCTACAGCAAGTGCTACGGTGCAGCCCGTGCCTCG	
			<b>Indel3</b>
NA93-11	358	CGGTGCAGGTGCCACCTTGTCGCCCGTCCGTCGCCGCCGCCGCCCGCCGCGCCAC	
OIL31	355	CGGTGCAGGTGCCACCTTGTCGCCCGTCCGTCGCCGCCGCCGCCCGCCGCGCCG.....CGCAC	
NA93-11	418	GACGCCGCCGCTCGTGGCGACGTTACCAACTACAAGCCGCTAGGGTGGAAAGTGCCAG	
OIL31	408	GACGCCGCCGCTCGTGGCGACGTTACCAACTACAAGCCGCTAGGGTGGAAAGTGCCAG	
NA93-11	478	TGCCGCGACCGCTGTTCGACCCCTGA	
OIL31	468	TGCCGCGACCGCTGTTCGACCCCTGA	

**B**

		<b>Indel1</b>	<b>Indel2</b>	<b>SNP1</b>
NA93-11	1	MRTAATPPL.AAAAAVAAVFLSALLL	ASASASASRLPPPRLLPLVGG	EVAVAVVAGEE
OIL31	1	MRTAATPPLAAAAVAAVFLSALLL.	ASASASRLPPPRLLPLVGG	EVAVAVVAGEE
			<b>Indel3</b>	
NA93-11	60	EKVR LGSSPPSCYSKYGCSPCVAVQVPTLSAPSVAAAA	AAARRRAARGDVHQLQAARV	
OIL31	59	EKVR LGSSPPSCYSKYGCSPCVAVQVPTLSAPSVAAAA	HDAAPLVATFTNYKPLGWKC	
NA93-11	120	EVPVPRPPVRPLTLRRARPVARRGVAWRVHGGARARALAVNYGVCGRVACPAAHGAALLL		
OIL31	119	QCRDLRFDP		
NA93-11	180	MLVVESLSSRRAERDC		

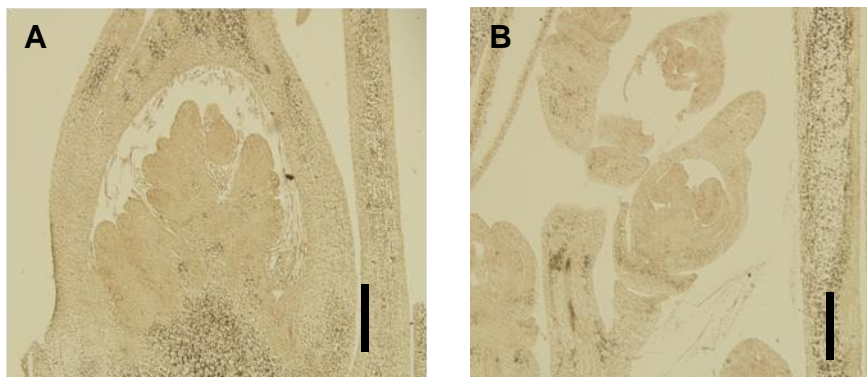
**Supplemental Figure 1**

*GAD1* sequence comparison between OIL31 and NA93-11. **(A)** Comparison of the genomic sequences in the *GAD1* coding region between NA93-11 and OIL31. **(B)** Comparison of the *GAD1* amino acid sequences between NA93-11 and OIL31. The red font indicates the different sites. Yellow rectangles represent Indels and SNPs between NA93-11 and OIL31.



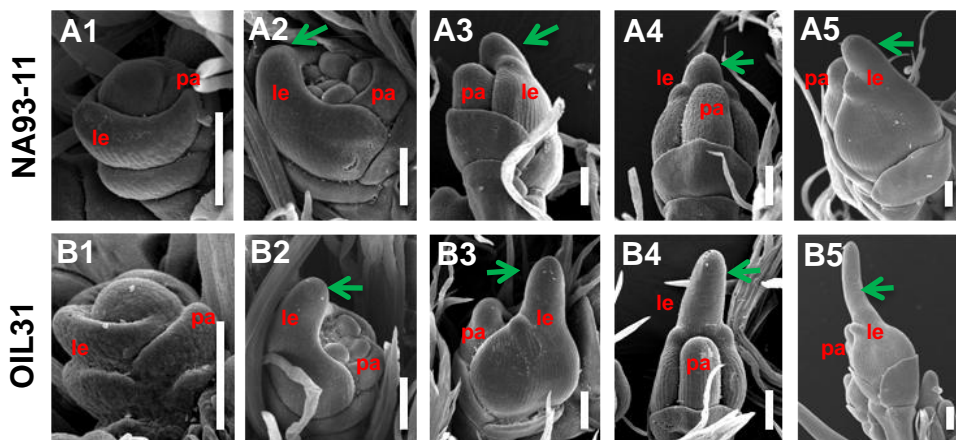
### Supplemental Figure 2

RNA *in situ* hybridization of *GAD1* in inflorescence meristem. **(A)** RNA *in situ* hybridization of *GAD1* at the primary branch formation stage in NA93-11. Bar = 50  $\mu$ m. **(B)** RNA *in situ* hybridization of *GAD1* at the secondary branch formation stage in NA93-11. Bar = 50  $\mu$ m. **(C)** RNA *in situ* hybridization of *GAD1* at the primary branch formation stage in OIL31. Bar = 50  $\mu$ m. **(D)** RNA *in situ* hybridization of *GAD1* at the secondary branch formation stage in OIL31. Bar = 50  $\mu$ m.



### Supplemental Figure 3

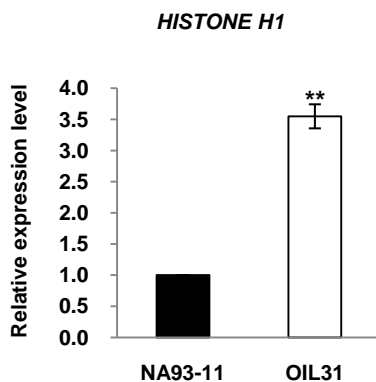
RNA *in situ* hybridization of the *GAD1* sense probe at different developmental stages. **(A)** Young panicle at the second branch formation stage. Bar = 100  $\mu\text{m}$ . **(B)** Young panicle at the spikelet developmental stage. Bar = 100  $\mu\text{m}$ .



#### Supplemental Figure 4

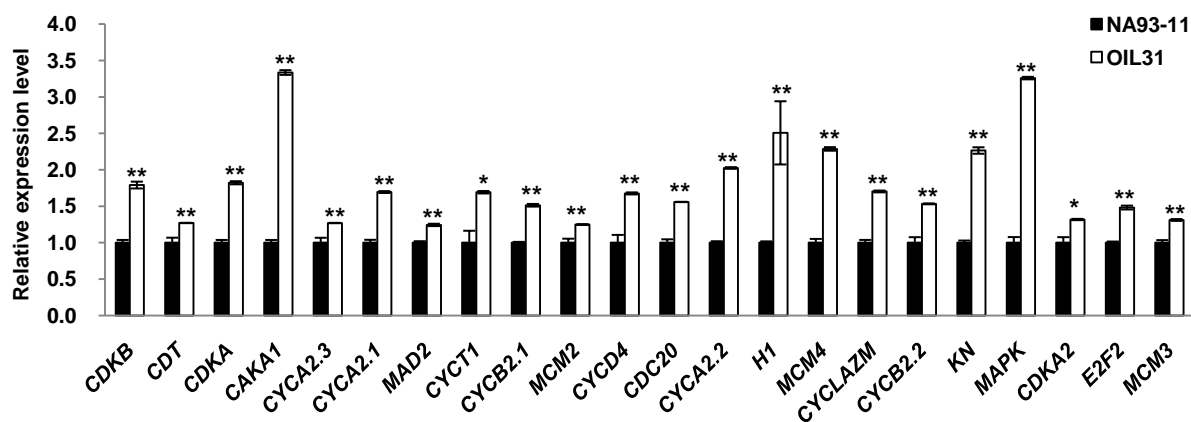
Scanning electron microscopy images of spikelets at different developmental stages. **(A1)** to **(A5)** Scanning electron microscopy images of spikelets at different developmental stages in NA93-11. Bar = 100  $\mu$ m. **(B1)** to **(B5)** Scanning electron microscopy images of spikelets at different developmental stages in OIL31. Bar = 100  $\mu$ m.

Green arrows point to the tip of lemmas.



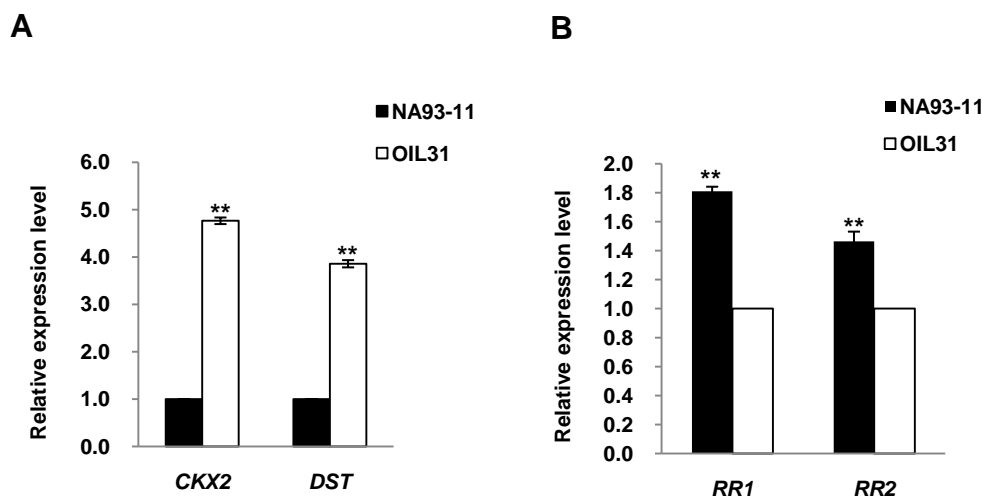
### Supplemental Figure 5

qRT-PCR analysis of *HISTONE H1* in young panicles of NA93-11 and OIL31. The data represent the average of three independent biological replicates. The statistical significance was set at  $P < 0.05$  based on a two-tailed Student's t test. Error bars represent the SD.



### Supplemental Figure 6

Transcript level comparison of 22 cell cycle-associated genes between NA93-11 and OIL31. The apices of glumes were subjected to qRT-PCR. The data represent the average of three independent biological replicates. The statistical significance was set at  $P < 0.05$  based on a two-tailed Student's *t* test. Error bars represent the SD.



### Supplemental Figure 7

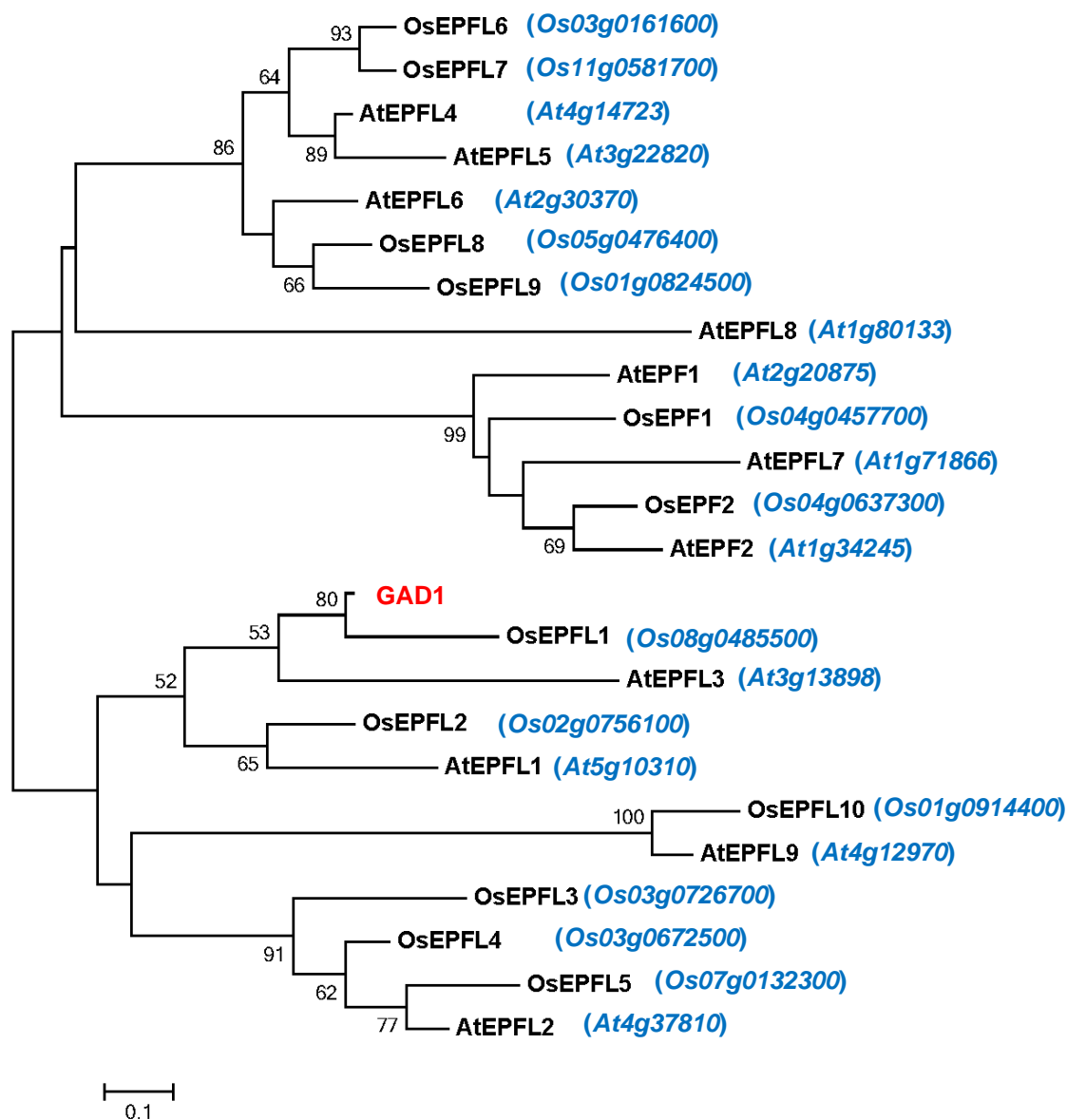
Comparison of *CKX2*, *DST* and *RR* transcript levels between NA93-11 and OIL31. **(A)** Comparison of *CKX2* and *DST* transcript levels in the young panicles of the NA93-11 and OIL31. **(B)** Comparison of *RR1* and *RR2* transcript levels in the young panicles of the NA93-11 and OIL31. The data represent the average of three independent biological replicates. The statistical significance was set at  $P < 0.05$  based on a two-tailed Student's *t* test. Error bars represent the SD.

1 attttgaccagaccacctcgtgcctctgctctgctcagtgctgctcactgcctcactcacactgtcacagt  
 73 gctcttctgggtataagtagctgggcgcgcggcgctcaccttcccttggctgcccgtgagcttcccgcgcgcgcatggc  
 151 acggcctcgtggcgctgggcgagcttcttggtaggcgaggtgctgccgggatcgagttgtctcgagagctactcagct  
 229 ATGAGGACGGCGGCCACGCCCTCTCGCCGCCGCCGCCGCCGCCGTCGCGGCAGTGTTCCTCTCTGCGTTG  
 1 M R T A A T P P L A A A A A A A V A A V F L S A L  
 304 CTGCTCGCCTCCGCCTCCGCCTCCAGGCTCCCTCCTCCTCGCCGCTTCTTCCCCTGGTTGGTGCGAGGTTGCG  
 26 L L A S A S A S R L P P P R R L L P L V G G E V A  
 379 GTGGCGGTGGTGGCTGGGAGGAGGAGAAGGTGCGGCTGGGGTCGAGCCCGCCGAGCTGCTACAGCAAGTGCTAC  
 51 V A V V A G E E E K V R L G S S P P S C Y S K C Y  
 454 GGGTGCAGCCCGTGCCTCGCGGTGCAGGTGCCACCTGTCCGCCCGTCCGTCCCCGCCGCCGCCGCGCAGAC  
 76 G C S P C V A V Q V P T L S A P S V P A A A A H D  
 529 GCCGCGCCGCTCGTGGCGACGTTACCAACTACAAGCCGCTAGGGTGAAGTGCCAGTGCCGCGACCGCCTGTT  
 101 A A P L V A T F T N Y K P L G W K C Q C R D R L F  
 613 GACCCCTGAacctgaggcgcgcgcgccccgtggcgcgcctggcgtggcgtggcgcgtgcatggcggggctcgcgc  
 126 D P \*  
 680 tcgcgctctcgtgtgaattacgggtgtgtgtggccgcgctcgcgtgcccggcagcacatggcgctgactgctgctgat  
 758 gctggtgggtggtggaatcgttgtcgtcgcgctcgggctgagaggattgttgatagattccgtgtaatatgccaggaca  
 836 aaattttgtcaccgctgctgctgcccatgcagctggatcgctcggctactctcaccacctatactgtatctttcact  
 914 ggcatctgctcgcgcttttggaaatctctcgggtgtggggttgcctcttgcattacacatgtttttcatgtatcgate  
 992 catgcctcctccatggaatctaattgggaatccatcatcgttcatgctggatggatggatggatgtagtgaatggtagt  
 1070 ttttcttatttttgttggagatggatatttttactttacatctaatacgatataatgttgtcttttaaattgagaatt  
 1148 tagc

### Supplemental Figure 8

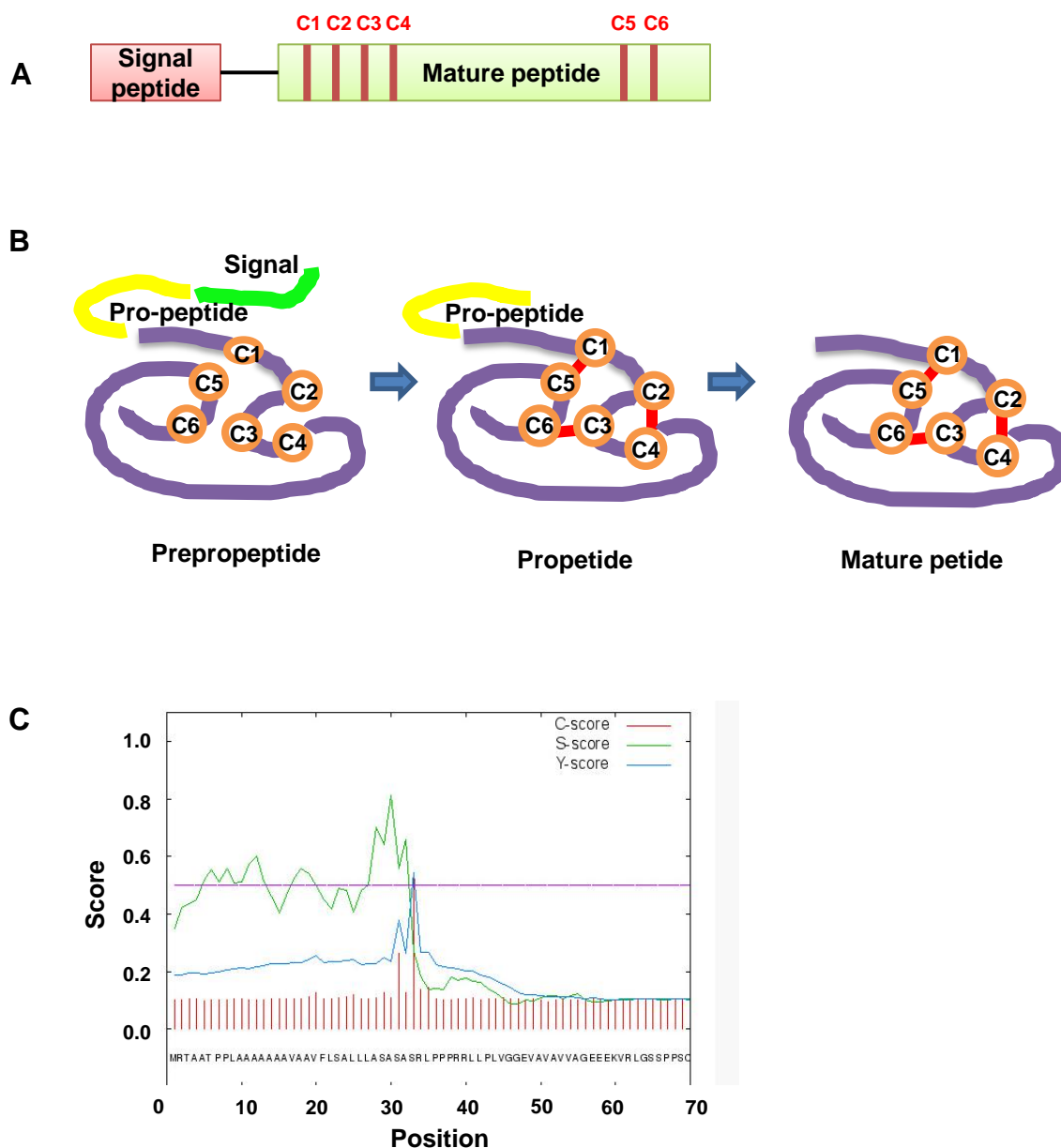
Full-length cDNA of *GAD1* and its predicted amino acid sequence. *GAD1* cDNA in W2014 is 1,151-bp long, containing an ORF of 384-bp, with 228-bp and 539-bp 5' and 3' untranslated regions, respectively, encoding a 127-amino-acid protein.





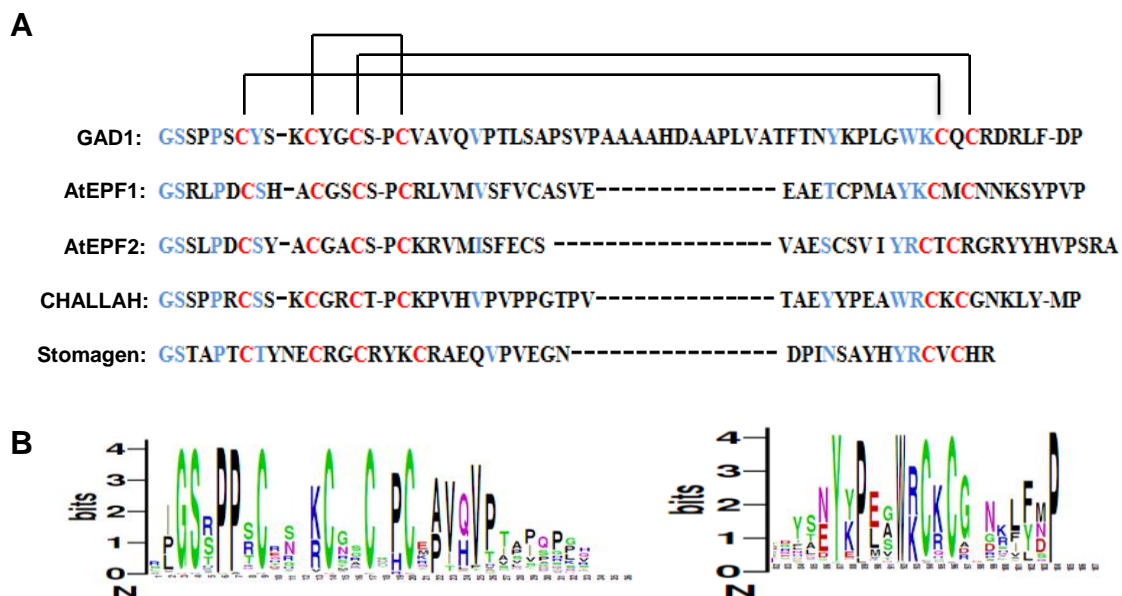
### Supplemental Figure 9

Phylogenetic tree of *EPF/EPFL* family genes from rice and *Arabidopsis*. Amino acid sequences were aligned using the ClustalX program. The phylogenetic tree was constructed by the MEGA 5 program. The evolutionary history was inferred using the Neighbor-Joining method. The percentage of replicate trees in which the associated taxa clustered together in the bootstrap test (1000 replicates) are shown next to the branches (values 50% or greater are shown). The tree is drawn to scale, with branch lengths in the same units as those of the evolutionary distances used to infer the phylogenetic tree. The evolutionary distances were computed using the JTT matrix-based method and are in the units of the number of amino acid substitutions per site.



### Supplemental Figure 10

Protein structure and maturation process predictions of GAD1. **(A)** Predicted protein structure of GAD1. The red rectangle represents the signal peptide; the green rectangle represents the mature peptide; red lines represent the consensus cysteine residues in GAD1. **(B)** Predicted GAD1 the maturation process. Red lines represent intramolecular disulfide bonds. **(C)** Analysis of the signal peptide in the GAD1 protein using SignalP4.1 (<http://www.cbs.dtu.dk/services/SignalP>).



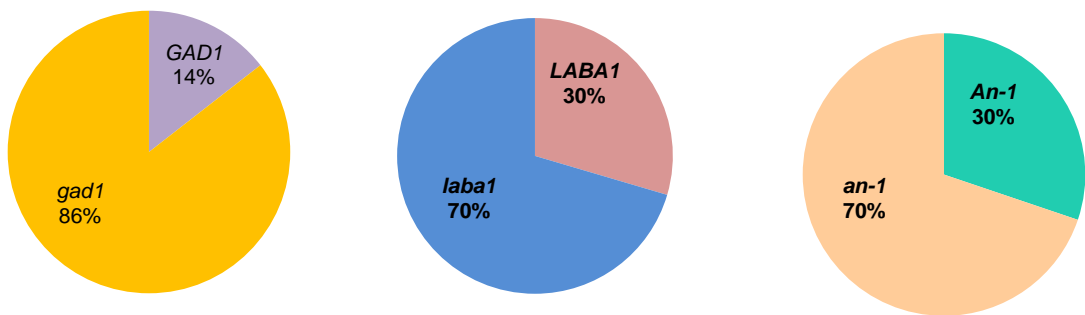
### Supplemental Figure 11

Analysis of the consensus sequence from the GAD1 protein. **(A)** GAD1 and other EPF/EPFL family member's mature protein sequences in *Arabidopsis* were aligned. The black lines represent the pairs of cysteine residues forming disulfide bonds predicted for the *EPF/EPFL* genes in *Arabidopsis*. The alignment uses putative active peptides based on the mature STOMAGEN sequence. AtEPF1: *At2g20875*; AtEPF2: *At1g34245*; CHALL: *At2g30370*; Stomagen: *At4g12970*. **(B)** Consensus sequences of the GAD1 protein. The consensus sequence graphically depicts conserved positions, and the height of the symbols indicates the relative frequency of each amino acid at that position.

Haplotype	The variation sites of <i>GAD1</i> coding region															No. of cysteine in protein	Awn phenotype	<i>Oryza rufipogon</i>	<i>indica</i>	<i>japonica</i>				
	Indel 1			Indel2						SNP 1	Indel3													
H1	G	C	C	-	-	-	-	-	-	T	-	-	-	-	-	-	-	-	-	6C	No awn	0	1	3
																					Awn	5	1	4
H2	-	-	-	-	-	-	-	-	-	G	-	-	-	-	-	-	-	-	-	6C	Awn	8	0	0
H3	-	-	-	G	C	C	T	C	C	G	-	-	-	-	-	-	-	-	-		Awn	1	0	0
H4	-	-	-	G	C	C	T	C	C	T	-	-	-	-	-	-	-	-	-		Awn	1	0	0
H5	-	-	-	G	C	C	T	C	C	G	C	G	C	C	G	C	C	G	C		6C	No awn	0	0
																				Awn		20	3	8
H6	-	-	-	-	-	-	-	-	-	G	C	G	C	C	G	C	C	G	C	6C	Awn	4	0	0
H7	G	C	C	-	-	-	-	-	-	G	C	G	C	C	G	C	C	G	C		Awn	4	0	0
H8	(G	C	C)2	G	C	C	T	C	C	G	C	G	C	C	G	C	C	G	C		Awn	3	0	0
H9	-	-	-	G	C	C	T	C	C	G	C	G	C	C	G	C	C	-	-	7C	No awn	0	93	34
																					Awn	6	2	8
H10	-	-	-	G	C	C	T	C	C	G	-	G	C	C	G	C	-	-	-	4C	No awn	0	13	21
																					Awn	1	5	4
																				Total	53	118	85	

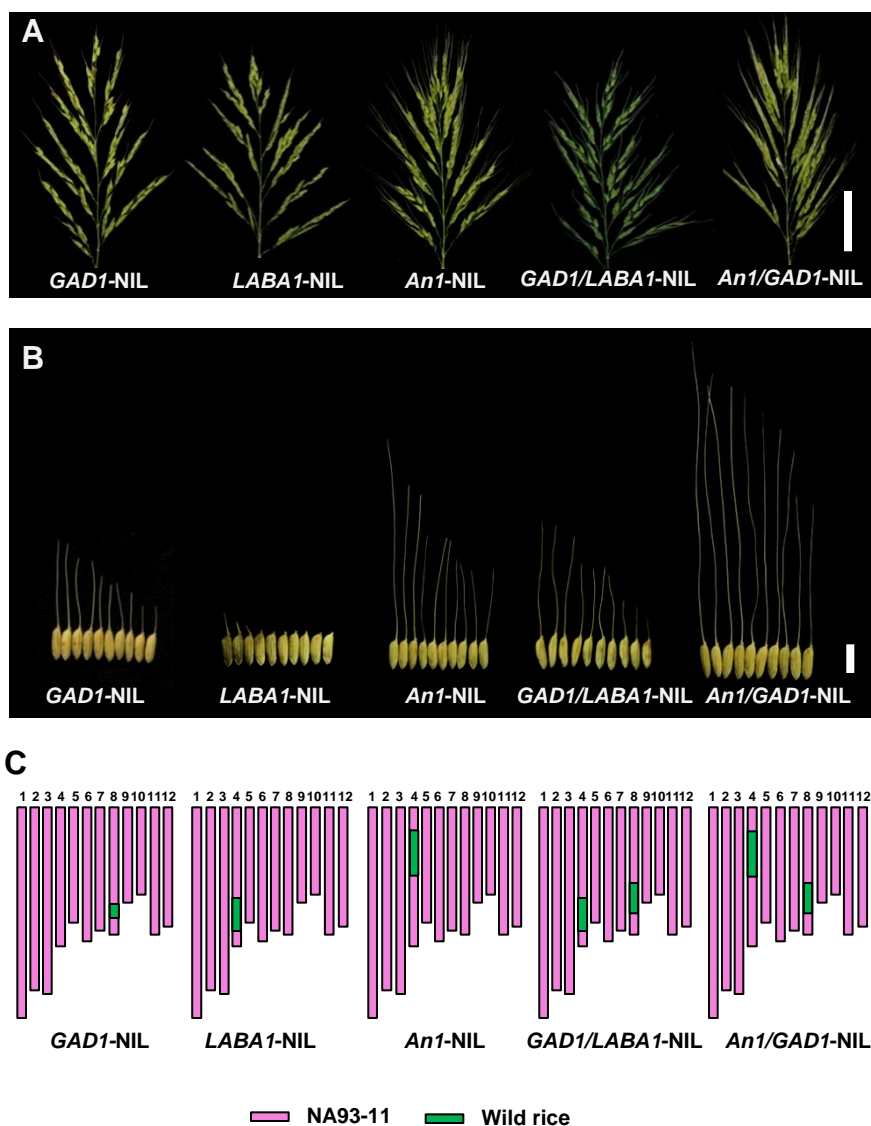
### Supplemental Figure 12

Haplotypes of *GAD1* in diverse wild and cultivated rice. The numbers of accessions that harbored each haplotype are indicated in the right-hand table.



### Supplemental Figure 13

Distribution of *GAD1/gad1*, *LABA1/laba1* and *An-1/an-1* in 159 varieties of cultivated rice.



### Supplemental Figure 14

Phenotypes of near-isogenic and pyramiding lines containing *GAD1*, *An-1* and *LABA1*. **(A)** Panicle comparison among *GAD1-NIL*, *LABA1-NIL*, *An1-NIL*, *GAD1/LABA1-NIL* and *An1/GAD1-NIL*. Bar = 50 mm. **(B)** Awn length comparison among *GAD1-NIL*, *LABA1-NIL*, *An1-NIL*, *GAD1/LABA1-NIL* and *An1/GAD1-NIL*. Bar = 10 mm. **(C)** Genotypes of *GAD1-NIL*, *LABA1-NIL*, *An1-NIL*, *GAD1/LABA1-NIL* and *An1/GAD1-NIL*.

**Supplemental Table 1.** The signal peptide and cleavage site predictions in the GAD1 protein.

Measure	Position	Value	Cutoff	signal peptide
max.C	33	0.542		
max.Y	33	0.544		
max. S	30	0.812		
mean S	1-32	0.517		
mean D	1-32	0.533	0.5	Yes

The prediction was performed by SignalP4.1 (<http://www.cbs.dtu.dk/services/SignalP>).

The GAD1 protein cleavage site was predicted to occur between positions of 32 and 33; D = 0.533; D-cutoff = 0.500.

**Supplemental Table 2.** *GAD1* nucleotide diversity and Tajima's *D* test.

Taxon	N	L	S	H	$\pi$	$\theta_w$	Tajima's <i>D</i>
<i>O. sativa</i>	203	579	20	23	0.00184	0.00663	-2.14422 (P<0.01)
<i>O. rufipogon</i>	53	540	25	30	0.00664	0.01289	-1.64418 (P>0.1)

N, total number of sequences; L, average length (bp) of the sequences per taxon; S, number of polymorphic (segregating) sites; H, number of haplotypes;  $\pi$ , average number of pairwise nucleotide differences per site calculated based on the total number of polymorphic sites;  $\theta_w$ , Watterson's estimator of per base pair calculated based on the total number of polymorphic sites.



**Supplemental Table 3. Primers used in this study.**

Purpose	Name	Forward primer (5'-3')	Reverse primer (5'-3')	
Mapping of <i>GAD1</i>	M12	AAGTTCTGAGCCCGTGAATG	CATCCGATCAACATCCATTG	
	M10	GAATACTGCAGCTGGGGTTG	TGACTTGATTATCATCAAATGTTC	
	M3	AAAAATGTGCACCAACTTCT	TAGAGGGCTCAAATTTGTGTT	
	M62	AATGTGTATTTAGATGCGATAGTTGTC	TTGATATCCGAAGCAATCC	
	M67	TCCAATCAAGAAACCAACCA	CCCTTCTTACCGTGTAGTCTCA	
	MX15	CTCTTGCCTCTTGAAGTTG	GCAAATGAGTTATGGCAATG	
	MX14	CTTCAAATTTATCTTTTTATTTCTACG	TCTGTTTTACCCTTCGATTCC	
	MX16	ATCTTCCACGCACGTTACAT	GCTACAAGCCCAAATGCTAC	
	MX12	GCCCATCTGCTACAGTTTGA	CCCCTCTCACTGACAGCTT	
	MX10	CAGCAAAGTCAAAGTGTAGC	GCAATTATTTATAGCTAGGAATTTAGA	
	MX9	TTCAGGAGTAACCCAATGTC	AACACCTCTGGTTTCTTGC	
	RM5485	ATGATTGCATCTGCATCACTGC	ATACGTGTTTCCAATGCGTAGC	
	RM256	GACAGGGAGTGATTGAAGGC	GTTGATTTGCGCAAGGGC	
	5'RACE	5'RACE inner	CAACGCAGAGAGGAACACTG	
		5'RACE outer	CACTTGCTGTAGCAGCTCG	
3'RACE	3'RACE inner	CGAGCTGTACAGCAAGTG		
	3'RACE outer	CAGTGTTCCTCTCTGCGTTG		
pCPL construct	1300-3	GGGGTACCTAGGCCCATGAAACAAGAAA	CGCGTCTGACTTGCAGCGATCTGAATAACA	
pRNAi construct	RNAi3	ATCACTAGTATGAGGACGGCGGCCACGCC	ATCGAGCTCCTAGCGGCTTGTAGTTGGTGA	
qRT-PCR	Actin	TGCTATGTACGTGCCATCCAG	AATGAGTAACCACGCTCCGTCA	
	<i>GAD1</i> -QRT	ATGCTGGTGGTGGTGAATC	TACATGCAAGAGGGCAACCCC	
	<i>OsCKX2</i> -QRT	CGGCAACAAGTGGGACAGTAA	CAGGGCGATGTAGGAAAGC	
	<i>DST</i> -QRT	ATCCAAGAAGGCAAGGTCAATC	ACACAGGAGGAAATTTGGAA	
	<i>OsRR1</i> -QRT	AGGATCAGCAGATGCATGAATG	GAGACGCTGTACGTCTTGTCTT	
	<i>OsRR2</i> -QRT	ACGATCTTCTCAAAGCCATCAAG	TGAGAGGCTTAAGGATGAAATCCT	
	<i>OsRR3</i> -QRT	GTTACGACGTTGGATAGTGG	ATTCTCCGACGACATTAGCA	
	<i>OsRR4</i> -QRT	CTGATCGACAGGAAGCTCAT	CAGGCATGCAGTAGTCAGTG	
	<i>MCM2</i>	AAGTTGGCAAAAGATCCACGG	CCCCAAACATAGCTAGTGCAA	
	<i>MCM3</i>	TTCATGCGTCACTAAATGCGAG	TGAATCTGGAAGCCCAATGTTC	
	<i>MCM4</i>	CCCGAATGCGATTCTCTGAA	ACCAGTGGCATGATCAGTTGC	
	<i>CYCB2.2</i>	CTCAAGGCTGCACAACTGACA	GCATTGACGGCTGGAATTTG	
	<i>CYCA2.2</i>	AGGTTGTCAAGATGGAGAGCGA	CGTTTTTGTCTTCTTGGA	
	<i>MAPK</i>	ACAGAGCAGCCGAATTTTGAGA	TTCAGCGAAGCTCACACTTGG	
	<i>KN</i>	CACGAGCTTCAAGAGATCGTGA	CCGGAATTGAGACACAATGTC	
	<i>CDC20</i>	TCAATCACCTGTTTGTGGC	TGGAGACAATCCAACGCAAAG	
	<i>CDKB</i>	AAGTTTGGCCAGGAGTGAGCA	TCAAGAGCATCAGCGTCGAGA	
	<i>CDKA2</i>	CGAGATTTGAAGCCCCAGAA	TCCGCGAGCTTCAATGAGTT	
	<i>CDT2</i>	AACCGCACCAACACTGGAA	GCAATTCACCATCTGCAGCTGG	
	<i>CDKA1</i>	GGTTTGGACCTTCTCTAAAATGC	AGAGCCTGTCTAGCTGTATCCTT	
	<i>CAK1A</i>	GACCGACAAGGGTTTCAGCAT	CCAGCATGTTTCAAGGAAGATACAAT	
	<i>CYCA2.3</i>	GTTTCGGTTGACGAGACGATGT	CGCTGCAAGGAACCTAGAAGCTG	
	<i>CYCT1</i>	GCATTTGTTGCAGCTCAAG	TCACCCTTCCGCTGACTTATTG	
	<i>CYCB2.1</i>	AAGTTTGGCCAGGAGTGAGCA	TCAAGAGCATCAGCGTCGAGA	
	<i>CYCD4</i>	GCCATGGAGTTGATACATCCAA	CCAGTAGGGCTCCGTGGAAT	
	<i>H1</i>	GCAAGGCACCTGACGCTT	AGGCAAGCCTTGTACAGATCCT	
	<i>CYClazm</i>	CACTCTCAAGCACCACACTGGA	ACAACCCTCAGCTTGTCTCAG	
	<i>CYCA2.1</i>	AGGTTGTCAAGATGGAGAGCGA	CGTTTTTGTCTTCTTGGA	
	<i>E2F2</i>	TGTTGGTGGCTGCCGATAT	CGCCAGGTGCACCCCTTT	
	<i>MAD2</i>	GAGCCATGCATATTCGACGTG	GGTGTGCAAGGAATGCGAGCTT	
	<i>GAD1</i> -CX	TTCTTGGTAGGCGAGGTGTC	CCTGGCATATTACAGCAATC	
	RNA <i>in situ</i> probe	<i>GAD1-in situ</i>	TTCCTGGTAGGCGAGGTGTC	CCTGGCATATTACAGCAATC
		<i>OsCKX2-in situ</i>	GTCCACGACGGCGAGCTCAA	CACCTCGCAGAAAACCAAGAT
		<i>OsRR1-in situ</i>	TGCTTGACGAATGAAGCAAC	AGGGCAAGGAAAAAGCTCTC
	Selective sweep analysis	<i>OsHistoneH4-in situ</i>	CTCCGCGACAACATCCAG	TCCAAACCTCCATATTTTCGGT
sweep1		TCACCTTTCTCCCAATTGC	GAGTGTCAACATGTGCAGC	
sweep2		TGGGCTTACGTTTTGGTTCA	GCCAAAGCAATTAGGGCAAT	
sweep3		AATCTGGTTGGTCTGTTGGTT	GGTATTTGGGTTAGGGCTGG	
sweep4		CCTGGTGAATACTTGTCCGGG	AATTTACTCCTCCGCGTTC	
sweep5		ACAAGCGGCTAATCGAATGT	GTGGCTGGAAAATCTCGTCT	
sweep6		ACCCTTCATGATGTTCTTGTGA	CAGAGCCTCAGAATTGCAGT	
sweep7		CTGCAAAAAGATGGACATGC	GACTGCTGCCACATCTAACA	
sweep8		GCTAGCACAATCGATCACCT	AGAAAGAGATGGATGCAGCG	
sweep9		GTTTACGGGCGGATTCTGTA	CCGTACTGACTGTCCATTG	
sweep10		TGGGGTACTACTTAGGTCCT	ATTGTAAGGCGTGTAAACC	
sweep11		GGCGGTTTGAATGTTGTGAG	ACCTCAAGCAAGCAAACAGA	
sweep12		CATTGATGTGGGCTCTCTGG	GGAGAACACCACCAAACGTA	
sweep13		TGCTCGCACAAGTCAATTTG	GGAATGAGCATCCCTACTGC	
sweep14		AAATCAACCGGTCAGACAC	GACAACGGCAACACTGAGTA	
sweep15	GGCTACATGGGCATTGAGAA	AGTCTGTTCTGAGGTGAGCT		