

**Supplemental Table 2.** DNA markers designed for determining wild-type and mutant alleles at the *E3* and *Ln* loci using a DNA sequencer

Marker name	PCR product size (bp)	Primer name	Primer sequence <sup>b</sup>
E3	<i>E3</i> (wild): 151	E3-F <sup>a</sup>	GGAGGGTATTGGATGATGC
	<i>e3</i> (mutant): 186	E3-Rwild	CCAATGTGAGAGCCTTTAGTTC
		E3-Rmutant	GATAAGAAAAGGAAAGAGAGACATG
Ln	<i>Ln</i> (wild): 130	Ln-ls-F	<b><u>CACGACGTTGTAAAACGAC</u></b> CTCCTTCTCTAAACCCTTTAAC
		Ln-ls-R	<b><u>CACGACGTTGTAAAACGAC</u></b> TTGGTATGATCATGAAGTTTACCTGA
	<i>ln</i> (mutant): 116	Ln-as-wild	CCAGAACGAAACCCCTCAG
	Ln-as-mutant	CATCGGGCAAATTGTTAAGGTG	
	M13-primer <sup>a</sup>	CACGACGTTGTAAAACGAC	

<sup>a</sup> Several nucleotides with a fluorescent material were attached to the 5' end of the primer. In this study, 6-FAM was used.

<sup>b</sup> Bold-typed nucleotides with underline indicate the M13-primer sequence.

**Supplemental Table 3.** Statistical analysis of single seed weight (SSW) and other seed yield components in the NsT-RIL population and their parental cultivars under Mito and Tsukuba experimental conditions

Trait	Experimental condition	Parental cultivars		Recombinant inbred lines (NsT-RILs, $n=181$ )							
		Natto-shoryu	Tachinagaha	ANOVA			Range	Mean	ANOVA		
				G	E	G:E			G	E	G:E
SSW (mg)	Mito	128 ± 2	365 ± 5	***	***	NS	145 — 339	228 ± 37	***	NS	***
	Tsukuba	148 ± 17	400 ± 13	(0.980)	(0.010)	(0.001)	161 — 326	229 ± 32	(0.753)	(0.000)	(0.099)
NS	Mito	576 ± 41	156 ± 24	***	NS	NS	99 — 567	229 ± 82	***	***	***
	Tsukuba	537 ± 148	153 ± 28	(0.854)	(0.002)	(0.002)	159 — 545	298 ± 64	(0.497)	(0.128)	(0.101)
NP	Mito	330 ± 18	89 ± 11	***	NS	NS	56 — 286	143 ± 36	***	***	***
	Tsukuba	296 ± 62	84 ± 16	(0.902)	(0.006)	(0.003)	79 — 258	153 ± 33	(0.533)	(0.014)	(0.115)
NSP	Mito	1.75 ± 0.04	1.76 ± 0.08	NS	NS	NS	0.97 — 2.08	1.58 ± 0.25	***	***	***
	Tsukuba	1.80 ± 0.13	1.82 ± 0.17	(0.007)	(0.047)	(0.001)	1.44 — 2.56	1.96 ± 0.21	(0.326)	(0.330)	(0.195)
NOP	Mito	2.18 ± 0.01	2.36 ± 0.03	***	NS	NS	1.89 — 2.70	2.28 ± 0.18	***	***	***
	Tsukuba	2.19 ± 0.03	2.33 ± 0.04	(0.874)	(0.003)	(0.009)	2.00 — 2.79	2.31 ± 0.19	(0.889)	(0.007)	(0.025)
RSS (%)	Mito	80.1 ± 1.7	74.3 ± 2.9	NS	NS	NS	40.7 — 87.6	69.5 ± 9.4	***	***	***
	Tsukuba	82.0 ± 6.3	78.0 ± 7.1	(0.156)	(0.052)	(0.005)	67.7 — 94.8	84.7 ± 5.1	(0.120)	(0.423)	(0.262)
SY (g)	Mito	74 ± 4	57 ± 9	**	NS	NS	22 — 110	51 ± 15	***	***	***
	Tsukuba	77 ± 12	61 ± 11	(0.440)	(0.021)	(0.000)	43 — 98	67 ± 12	(0.386)	(0.172)	(0.092)

SSW, single seed weight; NS, number of seeds per plant; NP, number of pods per plant; NSP, number of seed per pod; NOP, number of ovules per pod; RSS, rate of seed-set; SY, seed yield per plant.

G, E, and G:E represent the genotype, experimental condition, and the interaction between genotype and experimental condition, respectively, used in ANOVA.

Trait values for each genotype group is shown as mean ± standard deviation.

\*\* and \*\*\* represent statistical significance at  $P < 0.01$  and  $0.001$ , respectively, by ANOVA; NS = not significant.

Values in parentheses indicate the effect size ( $\eta^2$ ) of each factor calculated as a proportion of the sum of squared deviation of the factor to the total sum of squared deviation.

**Supplemental Table 4.** Correlation coefficients between seed yield components in the NsT-RIL population under Mito and Tsukuba experimental conditions (additional data for Table 1)

Trait	Experimental condition	SSW	NS	NP	NSP	NOP	RSS
NS	Mito	-0.548 ***					
	Tsukuba	-0.506 ***					
NP	Mito	-0.440 ***	0.919 ***				
	Tsukuba	-0.211 **	0.874 ***				
NSP	Mito	-0.492 ***	0.708 ***	0.392 ***			
	Tsukuba	-0.598 ***	0.205 **	-0.281 ***			
NOP	Mito	-0.396 ***	0.275 ***	0.076 NS	0.485 ***		
	Tsukuba	-0.511 ***	0.163 *	-0.231 **	0.835 ***		
RSS	Mito	-0.339 ***	0.649 ***	0.401 ***	0.870 ***	-0.004 NS	
	Tsukuba	-0.362 ***	0.153 *	-0.172 *	0.630 ***	0.101 NS	
SY	Mito	-0.071 NS	0.852 ***	0.820 ***	0.580 ***	0.090 NS	0.610 ***
	Tsukuba	0.157 *	0.757 ***	0.838 ***	-0.199 **	-0.199 **	-0.066 NS

SSW, single seed weight; NS, number of seeds per plant; NP, number of pods per plant; NSP, number of seed per pod; NOP, number of ovules per pod; RSS, rate of seed-set; SY, seed yield per plant.

\*, \*\*, and \*\*\* represent statistical significance at  $P < 0.05$ ,  $0.01$ , and  $0.001$ , respectively; NS = not significant.

**Supplemental Table 5.** Mapping of QTLs associated with single seed weight (SSW) and other seed yield components in the recombinant inbred line population derived from a cross between ‘Natto-shoryu’ and ‘Tachinagaha’ (NsT-RILs) at Mito and Tsukuba experimental conditions

Trait	Experimental condition <sup>a</sup>	Chromosome (Linkage group)	Position (cM)	Flanking marker	LOD score	Additive effect <sup>b</sup>	R <sup>2</sup>	Marker interval <sup>c</sup>	Designated QTL	
SSW (mg)	Mito (LOD > 3.3)	12 (H)	79.0	Sat_206	6.2	-12.3	0.112	Satt469 — Sat_206	<i>qSw12-1</i>	
		13 (F)	34.1	CSSR535	4.0	-9.4	0.064	Sat_039 — CSSR535	<i>qSw13-1</i>	
		17 (D2)	59.6	CSSR172	5.1	-10.9	0.087	Satt372 — CSSR172	<i>qSw17-1</i>	
		20 (I)	75.7	<i>Ln</i>	4.9	10.7	0.083	<i>Ln</i> — CSSR500	<i>qSw20-1</i>	
	Tsukuba (LOD > 3.2)	6 (C2)	22.4	Satt322	5.1	-8.7	0.071	Sat_153 — Satt322		
		12 (H)	70.3	Satt469	6.9	-10.3	0.102	GMES4012 — Satt469	<i>qSw12-1</i>	
		13 (F)	34.1	CSSR535	9.9	-12.4	0.148	Sat_039 — CSSR535	<i>qSw13-1</i>	
		17 (D2)	61.6	CSSR172	5.7	-8.9	0.078	Satt372 — CSSR172	<i>qSw17-1</i>	
		20 (I)	79.7	<i>Ln</i>	5.8	10.5	0.108	<i>Ln</i> — CSSR500	<i>qSw20-1</i>	
NS	Mito (LOD > 3.0)	12 (H)	77.0	Sat_206	5.4	24.3	0.088	Satt469 — Sat_206		
		19 (L)	137.9	<i>E3</i>	12.9	42.6	0.261	Sat_286 — <i>E3</i>		
		20 (I)	43.7	Satt614	3.1	-17.9	0.046	Satt419 — Satt614		
	Tsukuba (LOD > 3.1)	11 (B1)	111.9	Sat_123	5.1	19.6	0.092	Satt583 — Sat_123		
		12 (H)	70.3	Satt469	9.0	24.2	0.144	GMES4012 — Satt469		
		13 (F)	6.0	Sat_039	3.6	17.7	0.075	Sat_039 — CSSR535		
		19 (L)	130.9	Sat_286	5.9	22.6	0.123	Sat_286 — <i>E3</i>		
	NP	Mito (LOD > 3.0)	8 (A2)	138.4	Satt209	3.2	11.2	0.095	Satt329 — Satt209	
			12 (H)	77.0	Sat_206	5.1	11.5	0.100	Satt469 — Sat_206	
			19 (L)	136.9	<i>E3</i>	7.6	15.3	0.172	Sat_286 — <i>E3</i>	
Tsukuba (LOD > 3.2)		11 (B1)	94.5	Satt583	3.5	9.9	0.087	Satt519 — Satt583		
		12 (H)	73.0	Satt469	8.0	11.7	0.126	Satt469 — Sat_206		
		19 (L)	141.2	<i>E3</i>	10.7	14.3	0.181	<i>E3</i> — Satt373		
NSP	Mito (LOD > 3.2)	19 (L)	141.2	<i>E3</i>	12.5	0.115	0.202	<i>E3</i> — Satt373		
		20 (I)	74.7	<i>Ln</i>	6.6	-0.076	0.089	<i>Ln</i> — CSSR500		
	Tsukuba (LOD > 3.1)	13 (F)	34.0	CSSR535	12.4	0.089	0.168	Sat_039 — CSSR535		
		19 (L)	141.2	<i>E3</i>	7.5	-0.064	0.090	<i>E3</i> — Satt373		
		20 (I)	70.3	<i>Ln</i>	14.9	-0.104	0.235	Satt354 — <i>Ln</i>		
NOP	Mito (LOD > 3.3)	13 (F)	34.0	CSSR535	22.2	0.0834	0.218	Sat_039 — CSSR535		
		20 (I)	29.7	Satt419	3.3	-0.0276	0.022	Satt419 — Satt614		
		20 (I)	74.7	<i>Ln</i>	25.8	-0.0956	0.263	<i>Ln</i> — CSSR500		
	Tsukuba (LOD > 3.0)	13 (F)	34.0	CSSR535	21.8	0.084	0.188	Sat_039 — CSSR535		
		20 (I)	74.7	<i>Ln</i>	27.6	-0.111	0.304	<i>Ln</i> — CSSR500		
RSS (%)	Mito (LOD > 3.1)	12 (H)	76.0	Sat_206	4.4	2.43	0.065	Satt469 — Sat_206		
		19 (L)	141.2	<i>E3</i>	18.1	5.49	0.318	<i>E3</i> — Satt373		
	Tsukuba (LOD > 3.1)	19 (L)	141.2	<i>E3</i>	11.9	-2.46	0.231	<i>E3</i> — Satt373		
SY (g)	Mito (LOD > 3.1)	19 (L)	141.2	<i>E3</i>	12.9	7.53	0.238	<i>E3</i> — Satt373		
	Tsukuba (LOD > 3.2)	12 (H)	64.3	GMES4012	3.7	3.13	0.064	GMES4012 — Satt469		
19 (L)		141.2	<i>E3</i>	8.5	5.05	0.163	<i>E3</i> — Satt373			

SSW, single seed weight; NS, number of seeds per plant; NP, number of pods per plant; NSP, number of seed per pod; NOP, number of ovules per pod; RSS, rate of seed-set; SY, seed yield per plant.

<sup>a</sup> Values in parentheses represent the LOD threshold for the QTL detection by 1000-time permutation test at  $P = 0.05$ .

<sup>b</sup> Values indicate the contribution of the ‘Natto-shoryu’ allele on the trait estimated by the composite interval mapping (CIM) method.

<sup>c</sup> Genetic marker interval for which the LOD peak was located on the linkage map.