Supporting Information

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SI Materials and Methods

Plant Materials. Seeds of soybean cultivars Toyomusume and Hayahikari were provided by the Hokkaido Research Organization (HRO), Japan. The residually heterozygous line HC1-85H (1), which was heterozygous only around the *qPDH1* locus, was maintained at the NARO Hokkaido Agricultural Research Center (NARO/HARC). The 85R and 85S lines, which were selfed progeny lines of HC1-85H fixed for Hayahikari and Toyomusume genotypes, respectively, were also maintained at NARO/HARC. Accessions in the mini-core collection (Table S1) were obtained from the National Institute of Agrobiological Sciences (NIAS) in Japan. Other accessions were obtained from NIAS, the US Department of Agriculture (USDA), and soybean-breeding laboratories in Japan, including HRO, as described in Table S2. These accessions included "Norin (National-elite)" cultivars comprising Japanese "old cultivars" and "modern cultivars" released between 1939 and 1962 and between 1997 and 2007, respectively.

Growth Conditions. Nontransformed plants were cultivated in growth chambers under a regime of 15 h of light at 22 °C and 9 h of darkness at 17 °C as described previously (2). Recombinant inbred lines (RILs) derived from the cross Toyomusume × Jack were grown in the field under the same conditions as in our previous study (3). Transformants were raised in growth chambers as described elsewhere (4). Immature pods used for in situ hybridization were harvested 3 wk after anthesis whereas immature pods used for expression analysis of endocarp and other tissue after suture removal (Fig. 3*C*) were harvested 5 wk after anthesis, a stage at which the halves could be split by hand. Other immature pods used for ORF1-expression analysis were harvested 10 d after anthesis unless described otherwise. Leaves, stems, and roots were harvested from 3-wk-old plants. Immature seeds were harvested 3 wk after anthesis. After harvest, all samples were immediately frozen in liquid nitrogen and subsequently stored at −80 °C.

Evaluation of Shattering Resistance. Up to 20 fully matured pods were harvested from each plant and transferred to a chamber under conditions of 30% RH and ambient temperature. Samples of different genotypes were transferred simultaneously for comparison. Three weeks and 6 wk after transfer, the number of dehiscent and indehiscent pods was recorded for each genotype. As exceptions, the pods shown in Fig. 1 *A* and *C* were, respectively, stored under ambient humidity conditions (~40% and ~22% RH) in the laboratory before photographing. In addition, shattering resistance of RILs derived from the cross between Toyomusume and Jack was evaluated by heat treatment as described previously (5). The shattering scores presented in Table S2 were deposited in the Germplasm Resources Information Network (www.ars-grin.gov/) database as SHATLATE. The degrees of shattering resistance of the Japanese modern cultivars (Table S2) are cited from data deposited in the Japanese cultivars database of the Ministry of Agriculture, Forestry and Fisheries, Japan (www.hinsyu.maff.go.jp/).

Analyses of Pod Walls (Pod Halves) Torsion. Pod-wall torsion angles of two-seeded pods for 85R, 85H, and 85S plants or two- and three-seeded pods for Jack and transgenic plants were measured under the 30% RH condition with an instrument composed of two blades and a rod; one blade was freely movable along, and revolvable on, the rod whereas the other was fixed to the end of the rod. For measurements, the movable and fixed blades were attached to dehisced sides of distal and basal sections, respectively, of the pod wall. The torsion angle of the pod wall corresponded to the angle formed by the two planes of the blades and was determined with the Motic Images Plus 2.0S program (Shimadzu Rika) from images recorded with a digital camera (Power Shot A570; Canon) set vertically against the rod.

Fine Mapping of *qPDH1*. The *qPDH1* locus was previously determined to reside in a 134-kb region flanked by DNA markers Sca184-401k and Sca184-267k (6) on chromosome 16. A population of 2,535 self-pollinated progeny of HC1-85H were used to delimit the *qPDH1* locus using a previously described method (6) with the following modifications: Plants with recombinant genotypes were cultivated in a growth chamber instead of the field, and shattering resistance was evaluated at 30% RH, rather than by heat treatment. Briefly, plants with recombinant genotypes were self-pollinated, and resulting seeds fixed for the recombinant genotype were selected. The locus was mapped by analysis of phenotypes and graphical genotypes of these lines (Fig. S2). Primer sequences of DNA markers are listed in Table S3.

BAC Screening and DNA Sequencing. Primers for RT-PCR of the *Pdh1* gene were used to screen for BAC clones containing the *qPDH1* locus. BAC libraries were constructed using total DNA of the SS soybean cultivar Misuzudaizu after partial digestion with *Bgl* II or *Hin*d III (www.naro.affrc.go.jp/project/results/laboratory/harc/2004/cryo04-44.html).

Clone H88I22, from which amplified PCR products were obtained, was sequenced for the region flanked by markers Sca184_401k and Sca184_267k using a BigDye Terminator v3.1 cycle sequencing kit on a 3130xl Genetic Analyzer DNA sequencer (Life Technologies).

Genomic PCR and Direct Sequencing. To determine the genomic sequences of Hayahikari and Toyomusume, PCR was performed with LA Taq polymerase (Takara) as described previously (6). Primers were designed using the soybean genome sequence database (www.phytozome.net/soybean) and the Primer3 program (frodo.wi.mit.edu/). The 25-μL PCR mixture consisted of 200 ng of template DNA, 1× PCR buffer, 0.4 mM dNTP mixture, 0.2 μM forward and reverse primers, and 1.25 units of LA Taq polymerase. Amplification conditions consisted of 95 °C for 5 min, followed by 35 cycles of 98 °C denaturation for 10 s, 60 °C annealing for 30 s and 72 °C extension for 5 min, with a final step of 72 °C for 7 min. PCR products were separated on 0.8% (wt/vol) agarose, with amplified fragments of interest recovered from excised gel pieces using a QIAquick gel extraction kit (Qiagen). The isolated DNA was direct-sequenced using a DNA sequencer as described in *BAC Screening and DNA Sequencing*. To determine the genomic sequences of NIAS Japanese (www.gene.affrc.go.jp/databases-core_collections_jg_en.php) and world (www.gene.affrc.go.jp/databases-core_collections_wg_en.php) core accessions (7), PCR was performed with Pdh1seq primer pairs (Table S3) and GoTaq Hot Start Green Master Mix (Promega) following the manufacturer's protocol. Each template DNA was the same as reported previously (7). Amplification conditions consisted of 95 °C for 2 min, followed by 30 cycles of 95 °C denaturation for 30 s, 53 °C annealing for 30 s, and 72 °C extension for 90 s, with a final step of 72 °C for 5 min. After purification with ExoSAP-IT (Affymetrix), PCR products were sequenced using a BigDye Terminator v3.1 cycle sequencing kit on a 3500xl Genetic Analyzer DNA sequencer.

RNA Extraction and Analysis. An RNeasy plant mini kit was used in conjunction with on-column DNase I treatment according to the manufacturer's instructions (Qiagen) to isolate total RNA from plant tissues frozen in liquid nitrogen. The absence of genomic DNA contamination was confirmed by the lack of PCR amplification products using primers for *qPDH1*. First-strand cDNA was synthesized from 1 μg of total RNA using oligo(dT) primers and SuperScript III reverse transcriptase (Life Technologies). For semiquantitative analysis, RT-PCR was performed using first-strand cDNA as a template with gene-specific primers (Table S3) and AmpliTaqGold polymerase (Life Technologies), followed by agarose-gel electrophoresis to visually quantify PCR products. The same cDNA concentration was used for each sample. PCR conditions consisted of 95 °C preincubation for 5 min, followed by 27–36 cycles of 94 °C template denaturation for 30 s, 55 °C annealing for 30 s, and 72 °C extension for 30 s. Quantitative RT-PCR based on the SYBR Green I dye method was performed using first-strand cDNA as a template with gene-specific primers (Table S3) and an SYBR Premix Ex Taq II kit (Takara) on an ABI PRISM 7000 sequence detector (Life Technologies). Each of the two or three biological replicates was normalized using a cyclophilin gene (*CYP2*; TC224926) as an internal control (8). Amplifications were performed as follows: 95 °C preincubation for 3 min, followed by up to 40 cycles of 95 °C for 10 s, 55 °C for 15 s and 72 °C for 30 s. Standard curves were constructed using serially diluted cDNA.

The 5′ and 3′ Rapid Amplification of cDNA Ends. Full-length cDNA of ORF2 was isolated by 5′ and 3′ rapid amplification of cDNA ends (RACE). The 5′ RACE was performed using gene-specific primers (Table S3) and a 5′ RACE System for Rapid Amplification of cDNA Ends kit v2.0 (Life Technologies). cDNA synthesis for 3′ RACE was carried out as described in the previous section, with adapter-containing oligo(dT) primers (Table S3) used instead of regular oligo(dT) primers. The adapter primers and gene-specific primers (Table S3) were used to amplify cDNA 3′ ends.

Genotyping of Soybean Accessions for the SNP in *Pdh1*. Total DNA was isolated from seeds as described by Suzuki et al. (6) or Sayama et al. (9). PCR (94 °C for 5 min, followed by 35 cycles of 94 °C for 30 s, 60 °C for 30 s, and 72 °C for 30 s, and a final step of 72 °C for 7 min) was performed to amplify the SNP-containing region using the primers given in Table S3. The resulting products were purified using a MinElute PCR purification kit (Qiagen), followed by treatment with the restriction enzyme *Nhe* I (New England Biolabs). Digested samples were resolved by 2% or 3.5% agarose-gel electrophoresis. The following accessions were genotyped (Fig. 2*E*): SR cultivars Hayahikari, Harosoy, Wasekogane, Kariyutaka, Jack, Young, Yukihomare, and Tokei 992, and SS cultivars Toyomusume, Kitamusume, Enrei, Tachinagaha, Sachiyutaka, PI416937, Suzuyutaka, Shokukei 32, and Fukuyutaka (Table S2). For genotyping the SNP of the various accessions used for Fig. 4, another method based on "tetra-primer ARMS-PCR" (10) was also used (Fig. S3*E*). Primer sequences used are listed in Table S3.

QTL Analysis of RILs. A set of $F_{4:5}$ RILs was derived by single-seed descent from a cross between Toyomusume and Jack. Genomic DNA isolated from 126 F_4 plants was typed with five DNA markers on chromosome 16 (Fig. S4*B*). Shattering-resistance levels of F_5 lines were determined by heat treatment of pods harvested from mature field-grown plants as described previously (5). Simple interval mapping was performed with the recorded genotype and phenotype data using Windows Cartographer 2.5 (11) as described elsewhere (3).

Generation of Plasmid Constructs and Plant Transformation. A 3.4-kb genomic DNA fragment containing ORF2 from Toyomusume was amplified by PCR using forward and reverse primers (Table S3) according to the method described in *Genomic PCR and Direct Sequencing*. PCR products were cloned into a pCR-XL-TOPO vector (Life Technologies), followed by selection of inserts containing the desired sequence. Vectors with correct inserts were subsequently digested with *Eco*RI and *Sac* I, and the resulting fragment was cloned into a pUHR plasmid vector (4) (Fig. S4*A*). Biolistic transformation was performed using cultured cells derived from immature embryos of the cultivar Jack as described by Nishizawa et al. (4). T_0 plants regenerated from hygromycin-resistant, red-fluorescent embryogenic cells were grown to maturity. Each plant was evaluated for shattering resistance. Because the SS allele behaves as a partially or nearly completely dominant gene (Fig. 1*B*), T_0 plants were used for evaluation of pod dehiscence. Total DNA and RNA were isolated from leaves and 2- to 3-wk-old pod walls, respectively.

Alignment and Phylogenetic Analysis of the Pdh1 Protein. The predicted amino acid sequence of ORF2 was searched for conserved domains using the conserved domains search tool (www.ncbi.nlm.nih.gov/Structure/cdd/wrpsb.cgi) against the database CDD v3.12. To construct a phylogenetic tree based on dirigent proteins, several accessions were selected from Ralph et al. (12) and from previous studies of DIR protein or gene functions (13–17). Pdh1 and DIR protein sequences were aligned using ClustalX with default parameter values (Gonnet protein-weight matrix, gap penalty of 10.00, gap-length penalty of 0.20, and delay-divergent cutoff of 30%) (18). Phylogenetic analysis was then performed by neighbor joining using the MEGA6 program (19). The Jones–Taylor–Thornton substitution model and 1,000 bootstrap replications were used in the analysis (Fig. S5).

In Situ Hybridization. In situ hybridization was performed as previously reported by Kouchi and Hata (20) with a few modifications. Pods were cut into 2 × 5-mm strips, with the long side aligned with the fiber array, and fixed in FAA [5% (vol/vol) formalin, 5% (vol/vol) acetic acid, and 45% (vol/vol) ethyl alcohol] overnight at 4 °C. The samples were dehydrated in a graded ethanol series up to absolute ethanol and then a t-butanol series, and finally embedded in paraffin (Merck). Cross-sections (10 μm) across pod fiber layers were spread upon water on 3-aminopropyltriethoxysilane (APS)-coated glass slides (Matsunami) and dried overnight at 40 °C. For in situ hybridizations, samples were deparaffinized in D-limonene and rehydrated in a graded ethanol series up to water. Using primers listed in Table S3, a 400-bp fragment from *Pdh1* was amplified from genomic DNA using standard PCR conditions. The PCR fragment was subcloned into a pBluescript KS vector (Agilent Technologies). Digoxigenin (DIG)-labeled sense and antisense probes were prepared from the linearized plasmid by in vitro transcription with SP6 or T7 RNA polymerase using a DIG RNA labeling kit (Roche Diagnostics) according to the manufacturer's instructions.

Microscopy for Pod-Wall Lignification. Two- to 6-wk-old pods were examined for pod-wall lignification. Cross-sections (50 μm thick) were prepared by cutting the pods perpendicular to the pod-wall fiber orientation. Cross-sections were stained with phloroglucinol-HCl and observed under a microscope (E600; Nikon).

Moisture Determination of Pod Walls. Pod walls were transferred from 30% RH to 100% RH conditions, and then returned to 30% RH. Before each transfer, sample fresh weight was recorded after equilibrium was reached. Dry weight of samples was determined with an MA150 Infrared Moisture Analyzer (Sartorius). Moisture content of each sample was determined based on fresh and dry weights.

Determination of Lignin Content. Each pod wall sample was ground with a Wiley mill; the resulting meal was sieved to obtain a 40–80 mesh meal that was defatted in the usual fashion. This defatted meal was used for preparation of samples for lignin determination. Lignin content was obtained from the sum of acid-insoluble (Klason) lignin and acid-soluble lignin contents. Acid-insoluble lignin, with ash content ignored, was determined following Dence (21). Acid-soluble lignin content was obtained by spectrophotometric measurement of absorbance at 205 nm of clear supernatant derived from the acid-insoluble lignin analysis (22). The moisture content of the pod wall meal was determined before and after defatting based on a previously described method (23).

Statistical Analyses. Comparison of two means was performed using the Student *t* test whereas comparison of multiple means was carried out using Tukey's HSD method with PROC ANOVA or PROC GLM in SAS 9.3 (SAS Institute). Although raw values are shown in the figures, percentages of dehisced pods and relative transcript levels were arcsine and logarithmically transformed, respectively, before analysis. The exceptions were shattering scores and the shattering resistance of the accessions, which were compared between the resistant and the susceptible genotypes at *Pdh1*, and between the East Asian landraces and the North American cultivars, using the Mann–Whitney *U* test.

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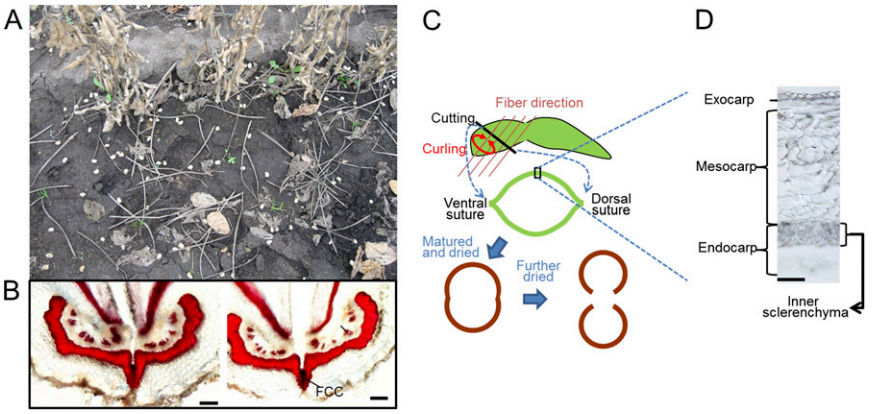

Fig. S1. Soybean pods and pod dehiscence. (*A*) Dehisced pods and shed seeds of an SS line in the field. (*B*) Light micrographs of cross-sections of mature soybean pod dorsal sutures. Figure courtesy of ref. 1. Pod halves (pod walls) seem to be bound tightly to each other by fiber-cap cells (FCCs) in both 85R [the NIL of the SR genotype (*Left*)] and 85S [the NIL of the SS genotype (*Right*)]. (Scale bar: 100 μm.) (*C*) Schematic illustration of a soybean pod wall. (Top) Whole pod wall, and fiber (red lines) and cutting (black line) directions; (middle) cutting plane and site of section; (bottom) cutting planes before and after pod dehiscence (left and right, respectively) at low humidity. (*D*) Cross-section of a 5-wk-old pod wall. (Scale bar: 100 mm.) Most of the inner part of the endocarp was so fragile that it collapsed during cross-section preparation. The highest cell density and the thickest cell walls are seen in inner sclerenchyma, suggesting the crucial role of this tissue in determining pod wall shape (see also Fig. 3*D*).

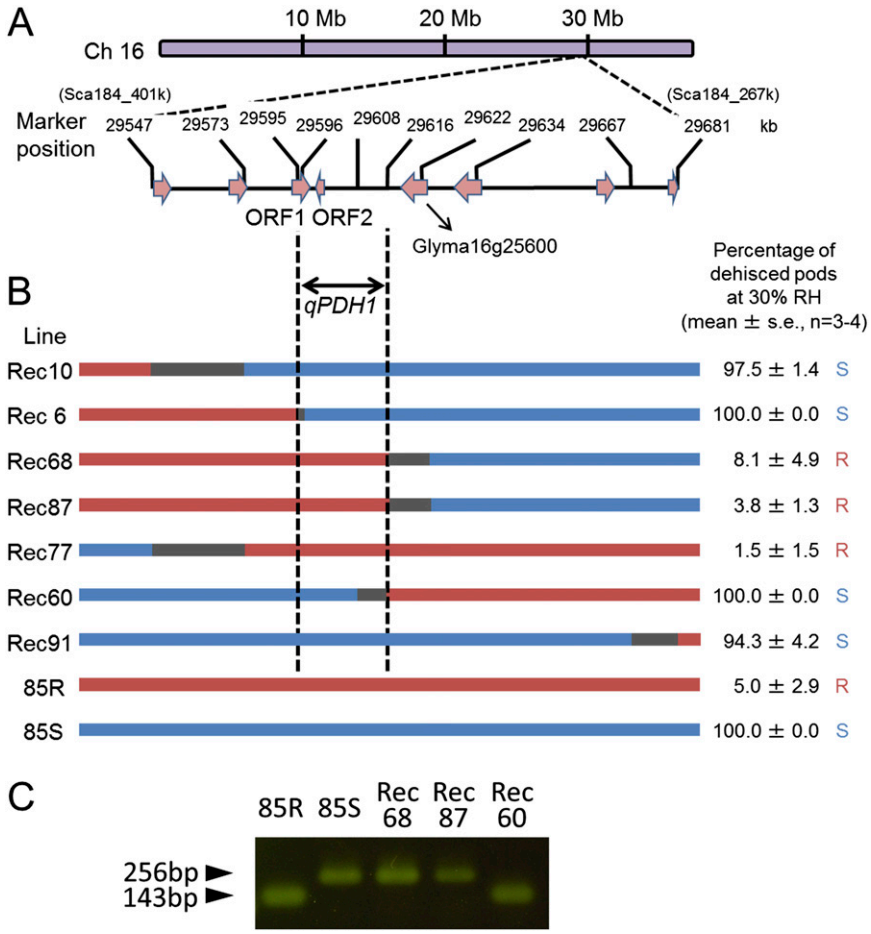

Fig. S2. Fine mapping of *qPDH1*. (*A*) Physical map and predicted ORFs (pink arrows) in a chromosome-16 134-kb genomic region flanked by Sca184_401k and Sca184_267k marker loci, onto which *qPDH1* had been previously mapped. (*B*) Graphical genotypes and degree of pod dehiscence of NILs derived from HC1-85H and carrying a recombination site in the 134-kb genomic region. Marker names and positions correspond to those in Table S3. The 85R and 85S are lines in which the genomic region was fixed for Hayahikari (resistant) and Toyomusume (susceptible) genotypes, respectively. Red and blue bars indicate genomic fragments derived from Hayahikari and Toyomusume, respectively; the gray bar indicates the occurrence of a recombination event somewhere in that region, as deduced from flanking marker genotypes. The genotype at the *qPDH1* locus, determined based on the degree of pod dehiscence (n = 3–4), is indicated for each line: *R* or *S*, corresponding to Hayahikari and Toyomusume genotypes, respectively. The candidate genomic region for *qPDH1* was localized to a 20-kb region flanked by 29595 and 29616 markers. (*C*) Indel polymorphism in Glyma16g25600 among several NILs used for fine mapping. Although the genotype with the deletion was characteristic of SR accessions in a previous study (24), lines with a gap in the present study were not necessarily SR.

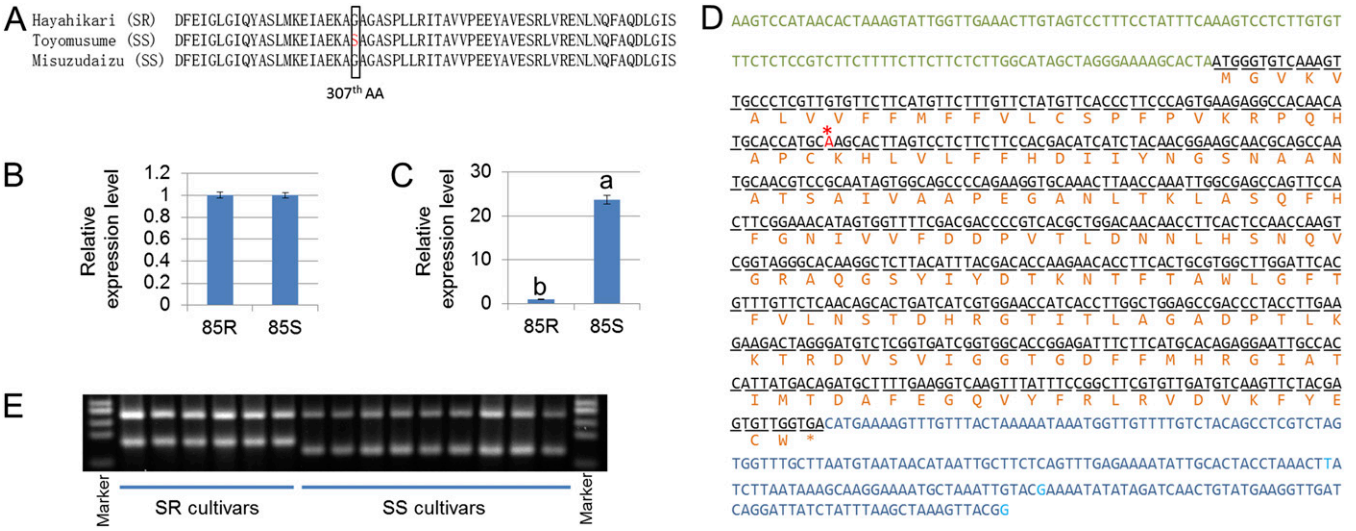

Fig. S3. Characterization of ORF1 and -2. (*A*) Alignment of peptide sequences predicted from partial ORF1 (Glyma16g25570.1; GRAS family transcription factor) sequences containing an amino acid substitution between Hayahikari (SR) and Toyomusume (SS). The predicted peptide sequence of Misuzudaizu (SS) is additionally aligned. The amino acid substitution site is boxed. (*B*) Comparison of relative ORF1 transcript levels between 85R and 85S. Expression levels were determined by quantitative RT-PCR using *CYP2* as a reference. Means and SE with two biological replicates are indicated by blue boxes and black bars, respectively. (*C*) Comparison of relative ORF2 transcript levels between 85R and 85S, determined by quantitative RT-PCR using *cyclophilin 2* (*CYP2*) as a reference (mean ± SE; $n = 2$). Different letters indicate significant difference ($P < 0.01$) between genotypes. (*D*) Full-length cDNA sequence and predicted amino acid sequence of *Pdh1* of Toyomusume (SS). Green, black, and blue letters represent 5′ UTR, coding, and 3′ UTR sequences, respectively. The SNP site conditioning shattering resistance is indicated by a red asterisk. Light-blue letters represent multiple sites followed by poly-A tails. The predicted amino acid sequence is represented by orange letters below the coding sequence. (*E*) Genotyping of ORF2 (*Pdh1*) by "tetra-primer ARMS-PCR." SR and SS genotypes yielded specific products of 169 bp and 146 bp, respectively, with a common band of 262 bp. Markers, ΦX174/*Hae*III. SR cultivars: Hayahikari, Harosoy, Wasekogane, Kariyutaka, Jack, and Yukihomare. SS cultivars: Toyomusume, Kitamusume, Enrei, Tachinagaha, Sachiyutaka, PI416937, Suzuyutaka, Shokukei 32, and Fukuyutaka.

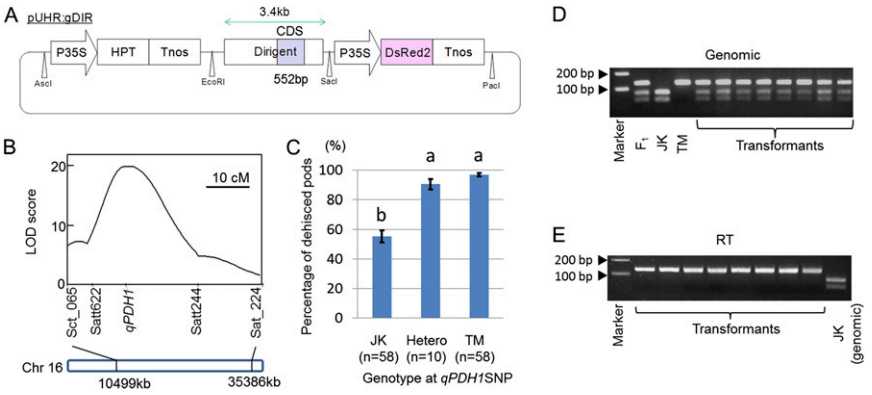

Fig. S4. Transformation of an SR cultivar, Jack (JK), with a functional *Pdh1* gene derived from the SS cultivar Toyomusume (TM). (*A*) Plasmid construct used for biolistic transformation. (*B*) Interval mapping of pod dehiscence on chromosome 16 in an F_4 population derived from the cross Toyomusume × Jack. (*C*) Degree of pod dehiscence of F_4 lines according to genotype at *qPDH1*. Bars represent SE. (*D*) *Nhe* I digestion of PCR products containing the SNP of *qPDH1* in the genomic template. Plants selected for both hygromycin resistance and red fluorescence carried the ORF2 sequence from Toyomusume. (*E*) *Nhe* I digestion of PCR products containing the SNP of *qPDH1* in the cDNA template. All transformants expressed transcripts of ORF2 in their pod walls. Digested PCR product from the Jack genomic template was used as a reference.

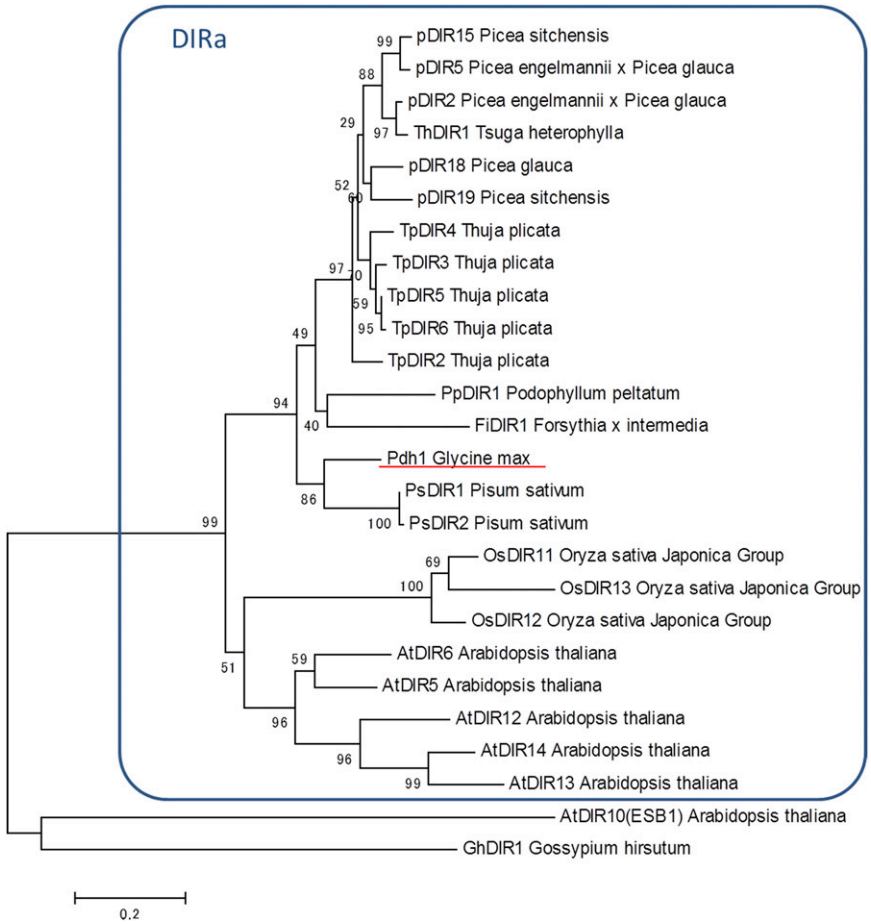

Fig. S5. Phylogenetic tree derived from analysis of predicted amino acid sequences of Pdh1 and dirigent(-like) protein sequences belonging to the DIRa subfamily published in Ralph et al. (12) and DIR proteins associated with lignin deposition. Protein sequences were retrieved from the National Center for Biotechnology Information (NCBI). DIR nomenclature follows Ralph et al. (12). Except for Pdh1, deduced amino acid sequences of the 56 dirigent-like protein genes predicted in the soybean genome assembly (Glyma1 by Phytozome) are not included in the tree. Although a genomic region on chromosome 2 is highly similar to the region carrying *pdh1* on chromosome 16, no ORF is predicted in this region in Glyma1.

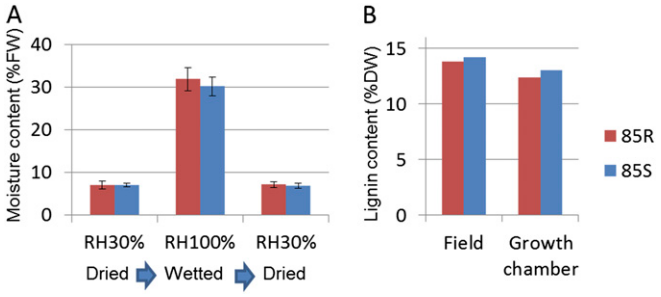

Fig. S6. Comparison of pod-wall characteristics of NILs for *Pdh1*. (*A*) Moisture content of pod walls of 85R and 85S. Pod-wall weights were measured after stabilization at each humidity condition. Pod walls were then dried at 105 °C, with weights and moisture content subsequently determined. (*B*) Lignin content of pod walls of 85R and 85S. Analysis of variance revealed a significant effect ($P < 0.05$) of growth environment but not of genotype.

Table S1. Soybean accessions of the mini-core collection and their sequences of *Pdh1*

Accession no.*	Accession name	Country	Sequence†
Japanese landraces			
JP28880	KITAJIRO	Japan	S-t
JP29621	KANAGAWA WASE	Japan	S-t
JP28507	JU.K.KOKU	Japan	S-t
JP28214	KUROGOYOU	Japan	S-t
No. N/A	KOITO	Japan	S-t
JP30135	KURODAIZU(AO HIGUU CHUU)	Japan	S-t
JP53292	YAKUMO MEAKA	Japan	S-t
JP73172	NATTOUMAME	Japan	S-m
JP28922	KOIBUCHIMURA ZAIRAI	Japan	S-t
JP28869	DATE CHA MAME	Japan	S-t
JP27922	TAKIYA	Japan	S-t
JP28474	SHAKKIN NASHI	Japan	S-t
JP27920	AKITA ANI	Japan	S-t
JP35409	HIKU ANDA	Japan	S-m
JP27948	FU.K.UI SHIRO	Japan	S-t
JP29500	KISAYA(NATSU)	Japan	S-t
JP27900	ABURA MAME	Japan	S-t
JP28996	SHAKUJOU MAME	Japan	S-m
JP29210	YAHAGI	Japan	S-t
JP28252	SOKOSHIN	Japan	S-t
JP28947	SHIMO HISAKATA DAIZU	Japan	S-t
JP73030	KOMAME	Japan	S-t
JP67658	AZEMAME	Japan	S-t
JP76512	AOBAKO	Japan	S-t
JP28049	MEGURO 1	Japan	S-t
JP27974	OOJIRO	Japan	S-t
JP28770	HOUJAKU	Japan	S-t
JP29253	ZAIRAI 51–2	Japan	S-t
JP27890	CHADAIZU	Japan	S-t
JP28914	IHHON SANGOU	Japan	S-m
JP76514	HITORIMUSUME	Japan	S-t
JP29346	AKASAYA	Japan	S-m
JP85549	KURUMIMAME	Japan	S-t
JP29213	HIME DAIZU	Japan	S-t
JP28320	AKUDEN SHIRAZU	Japan	S-t
JP67662	AOAKIMAME	Japan	S-t
JP110355	DAIZU	Japan	S-t
JP29207	CHUU TEPPOU	Japan	S-t
JP87838	DADACHAMAME	Japan	S-t
JP27891	KUROTOME	Japan	S-t
JP27863	KUROHIRA	Japan	S-t
JP29217	NAKAHATA ZAIRAI	Japan	S-m
JP29389	AKA DAIZU	Japan	S-t
JP76302	AMAGI ZAIRAI 90D	Japan	S-t
JP73149	KUROMAME	Japan	S-t
JP29408	COL/EHIME/1983/UTSUNOMIYA 22	Japan	S-t
JP208667	KURAKAKE	Japan	S-t
JP76321	MAETSUE ZAIRAI 90B	Japan	S-m
No. N/A	COL/TANBA/1989/ODAGAKI 2	Japan	S-t
JP27858	SHIRATAMA	Japan	S-t
JP28489	KOSA MAME	Japan	S-t
JP29363	KOKUBU 7	Japan	S-t
JP76560	HITASHIMAME	Japan	S-t
JP29413	COL/EHIME/1983/UTSUNOMIYA 28	Japan	S-t
JP29427	COL/EHIME/1983/UTSUNOMIYA 37	Japan	S-t
JP70093	MOCHI-DAIZU	Japan	S-t
JP28377	KUMAJI 1	Japan	S-t
JP29715	ITSU.K.I ZAIRAI 83H	Japan	S-t
JP29696	NANKAN ZAIRAI 83	Japan	S-t
JP28433	HAI MAME	Japan	S-t
JP29292	SAGA ZAIRAI	Japan	S-t
JP29493	KOMUTA	Japan	R

Table S1. Cont.

Accession no.*	Accession name	Country	Sequence†
JP29533	BAN KURO DAIZU	Japan	S-t
JP27579	GIN DAIZU	Japan	R
Jananese others			
JP28862	ENREI	Japan	S-t
JP29161	NATTOU KOTSUBU	Japan	S-m
JP27439	TOKACHI NAGAHA	Japan	S-t
JP29559	AKISENGOKU	Japan	S-t
JP29668	FU.K.UYUTAKA	Japan	S-t
JP53267	SHIZUNAIDAIZU	Japan	S-t
JP29149	CHIZU.K.A IBARAKI 1	Japan	S-t
JP53264	OOYACHI 2	Japan	S-t
JP37744	ONI HADAKA	Japan	S-t
JP27886	MIYAGI SHIROME	Japan	S-t
JP227175	KURODAIZU(GEIHOKU)	Japan	S-t
JP29184	TAMAHOMARE	Japan	S-t
JP67990	HIMESHIRAZU	Japan	S-t
JP227399	COL/EHIME/1–2	Japan	S-t
JP67989	TSURUSENGOKU	Japan	S-t
Chinese landraces			
JP28911	MANSHUU	China	S-t
JP28415	RIGAI SEITOU	China	R
JP30007	PEKIN DAI OUTOU	China	S-t
JP27605	MASSHOKUTOU(KOU 502)	China	S-t
JP30090	ICHIGUUHOU	China	R
JP27605	MASSHOKUTOU(KOU 503)	China	S-t
JP30071	CHOYOUTOU	China	R
JP28432	PEKING	China	R
JP35718	ANTO SHOU.K.OKUTOU	China	R
JP30105	BONGCHUNBAEKJAM	China	S-t
JP30072	SENYOUTOU	China	R
JP27543	HAKKA ZASHI	China	R
JP27584	KARASUMAME	China	S-t
JP30025	HAKUBI	China	R
JP28298	AOKIMAME	China	R
JP29613	MANSHUU MASSHOKUTOU	China	R
JP35719	HAKUCHIKOU	China	R
JP30032	BISHUU DAIZU	China	S-t
Korean landraces			
JP28912	CHOUSENSHU(CHA)	Korea	S-m
JP27956	NEZUMI META	Korea	S-m
JP27587	SHIROSOTA	Korea	S-t
JP29496	URONKON	Korea	S-t
JP29781	SEITA	Korea, South	S-t
JP35506	KLS 203	Korea, South	S-t
JP29774	CHUUHOKU 2	Korea, South	S-t
JP35462	CHIENEUM KONG	Korea, South	S-m
JP29903	KONGNAMUL KONG	Korea, South	S-t
JP29881	OKJO	Korea, South	S-t
JP29762	HEAMNAM	Korea, South	S-t
JP29803	HEU.K.DAELIP	Korea, South	S-t
JP29789	CHEONGYE MYONGTAE	Korea, South	S-t
JP29827	KEUMDU	Korea, South	S-t
JP29819	JEOKGAK	Korea, South	S-t
JP29882	OUDU	Korea, South	R
JP29794	GAPSANJAELAE(I)	Korea, South	R
SE Asian landraces			
JP30174	SANDEK SIENG	Cambodia	R
JP43384	BARITOU 3 A	Indonesia	R
JP30207	LOCAL VAR(SEPUTIH RAMAN)	Indonesia	S-t
JP30206	PETEK	Indonesia	S-m
JP30216	JAVA 5	Indonesia	S-t
JP30213	JAVA 7	Indonesia	S-m

Table S1. Cont.

Accession no.*	Accession name	Country	Sequence†
JP30210	LOCAL VAR.(TEGINENENG)	Indonesia	S-t
JP35796	KS 1034	Malaysia	S-t
JP35788	KE 32	Philippines	R
JP35787	L 2A	Philippines	S-t
JP49014	MISS 33 DIXI	Philippines	S-t
JP30146	KARASUMAME(HEITOU)	Taiwan	R
JP31111	POCHAL	Taiwan	S-m
JP30138	KARASUMAME(SHINCHIKU)	Taiwan	S-t
JP30147	KARASUMAME(NAIHOU)	Taiwan	S-t
JP38385	COL/THAI/1986/THAI-78	Thailand	R
JP38386	COL/THAI/1986/THAI-80	Thailand	R
JP30176	CHIENGMAI PALMETTO	Thailand	S-m
JP30179	SAN SAI	Thailand	S-t
SE Asia, others			
JP30196	MERAPI	Indonesia(B)	S-m
JP30197	RINGGIT	Indonesia(B)	S-m
South Asian landraces			
JP30272	PK 73–54	India	R
JP30258	M 581	India	H
JP30238	U 1416	India	R
JP30335	N 2295	India	R
JP30349	BHATMAS	India	R
JP30311	COL/PAK/1989/IBPGR/2326(1)	India	R
JP30303	M 44	India	S-t
JP30409	M 918	India	R
JP30363	HM 39	India	S-t
JP40391	M 42	Nepal	S-t
JP40444	U 1042–1	Nepal	R
JP74676	U 1290–1	Nepal	R
JP40354	U 8006–3	Nepal	R
JP40382	COL/PAK/1989/IBPGR/2323(2)	Nepal	R
JP40403	N 2392	Nepal	R
JP40452	N 2491	Nepal	S-t
JP40464	L 317	Nepal	R
JP84093	M 652	Nepal	R
JP30419	U-1741–2-2 NO.3	Nepal	R
JP40374	KADI BHATTO	Nepal	S-t
JP74682	E C 112828	Pakistan	R
JP74681	U 1155–4	Pakistan	R
Others			
JP30465	Fiskeby V	Sweden	S-t
JP31043	Williams 82	USA	R

N/A, not available; SE, South East.

*Accessions without numbers are not currently publicly available from the NIAS GenBank.

†Sequences "S-t", "S-m," and "R" are the same as those of Toyomusume, Misuzudaizu, and Hayahikari, respectively. "S-t" and "S-m" are of the SS genotype whereas "R" is of the SR genotype.

Table S2. Soybean accessions and their genotypes at the SNP of *Pdh1* and shattering scores

Accession no.*	Accession name	Province	Country	*Pdh1*†	Shattering score‡
Landraces (East Asia)					
PI089138	Zontanoruk-on	Hamgyong Puk	Korea, North	R	—
PI097094	N/A	Hwanghae Puk	Korea, North	R	1.0
PI398296	N/A	Kyonggi	Korea, South	S	3.0
PI 399043	N/A	Cheju	Korea, South	S	3.0
PI 407801	N/A	Kyonggi	Korea, South	S	2.0
PI 407849	N/A	Cholla Puk	Korea, South	S	3.5
PI 408342	N/A	Cheju	Korea, South	S	3.5
PI 423954	Shirome	Kumamoto	Japan	S	—
PI 423967	Nabeshima	Kumamoto	Japan	S	—
PI 424391	N/A	Cholla Puk	Korea, South	S	2.5
PI 567258	He pi dou	Jiangxi	China	S	4.0
PI 567293	Ben di huang dou	Gansu	China	R	1.0
PI 567298	Chan yao dou	Gansu	China	R	2.5
PI 567368	Xi he huang dou	Ningxia	China	R	1.0
PI 567395	Lai wa dou	Shaanxi	China	S	1.0
PI 567481	Bao ding huang dou	Hebei	China	R	1.0
PI 567503	Niu mao huang	Hebei	China	R	1.5
PI 567525	Cao qing huang dou	Shandong	China	R	2.5
PI 567700	Fu yang (19)	Anhui	China	R	1.5
PI 587552	Nan jing da ping ding huang yi 1	Jiangsu	China	R	4.0
PI 587666	Er dao zao	Anhui	China	R	2.5
PI 587752	Xian ning dong huang dou jia	Hubei	China	R	2.0
PI 587799	Wu chang zao huang dou	Hubei	China	R	2.0
PI 587946	Ping nan qiu da dou	Fujian	China	S	—
PI 588000	Shi yue huang	Sichuan	China	S	—
PI 588047	Huang ke wu dou	Guangdong	China	R	—
PI 588053A	Xiao li huang	Guangdong	China	S	4.5
PI 594451	Liu yue bao	Sichuan	China	S	3.5
PI 594554	Huang pi tian dou	Jiangxi	China	S	—
PI 594579	Zhong he tian cheng dou	Hunan	China	R	3.5
PI 594597	Ning yuan ba yue huang	Hunan	China	S	—
PI 594615	Liu yue zao	Guizhou	China	R	2.0
PI 594629	Xiao hua lian	Guizhou	China	S	4.0
PI 594770A	Fu sui chang ping hei dou	Guangxi	China	R	—
PI 594773	Fu sui qu li dou	Guangxi	China	R	—
PI 594777	Liu yue huang	Yunnan	China	S	3.5
PI 594788	Da zao dou	Yunnan	China	S	—
PI 602991	Niu jiao qi da hei dou	Shandong	China	R	3.5
PI 603318	N/A	Heilongjiang	China	R	2.5
PI 603336	N/A	Heilongjiang	China	R	2.5
PI 603357	N/A	Jilin	China	R	1.0
PI 603384	N/A	Jilin	China	R	2.5
PI 603420	N/A	Nei Monggol	China	R	3.5
PI 603424A	N/A	Nei Monggol	China	R	—
PI 603516	N/A	Shaanxi	China	S	2.5
PI 603596	N/A	Fujian	China	S	5.0
PI 603675	N/A	Jiangsu	China	S	2.5
Old North American cultivars					
PI548362	Lincoln	Unknown	Unknown	R	—
PI 548379	Mandarin (Ottawa)	Heilongjiang	China	R	—
PI 548445	CNS	Jiangsu	China	S	1.0
PI 548406	Richland	Jilin	China	R	—
PI 548488	S-100	Missouri	USA	R	2.5
PI 548477	Ogden	Tennessee	USA	R	2.0
PI 548298	AK [Harrow]	Unknown	China	R	—
PI 548318	Dunfield	Jilin	China	R	—
PI 548391	Mukden	Liaoning	China	R	—
PI 548657	Jackson	North Carolina	USA	R	1.0
PI 548348	Illini	Unknown	China	R	—
PI 548485	Roanoke	Jiangsu	China	R	1.0
PI 548311	Capital	Ontario	Canada	R	—
PI 548603	Perry	Indiana	USA	R	—

Table S2. Cont.

Accession no.*	Accession name	Province	Country	*Pdh1*†	Shattering score‡
PI 548382	Manitoba Brown	Liaoning	China	S	—
PI 548456	Haberlandt	Pyongyang	Korea, North	S	2.5
FC 33243	Anderson	Unknown	Unknown	R	—
Modern North American cultivars					
PI 556511	A3127	Michigan	USA	R	—
PI 533655	Burlison	Illinois	USA	R	1.0
PI 548512	Century	Indiana	USA	R	1.0
PI 525453	Conrad	Iowa	USA	R	1.0
PI 508083	Dassel	Minnesota	USA	R	—
PI 513382	Glenwood	Minnesota	USA	R	—
PI 553047	Gordon	Georgia	USA	R	1.0
PI 540552	Hoyt	Ohio	USA	R	1.3
PI 518664	Hutcheson	Virginia	USA	R	1.0
PI 548985	Kershaw	South Carolina	USA	R	2.0
PI 533602	Lloyd	Arkansas	USA	R	1.0
PI 548643	Maple Glen	Ontario (Ottawa)	Canada	R	—
PI 548638	OAC Libra	Ontario (Guelph)	Canada	S	—
PI 548644	OAC Musca	Ontario (Guelph)	Canada	R	—
PI 515961	Pennyrile	Kentucky	USA	R	1.8
PI 536637	Perrin	South Carolina	USA	R	1.5
PI 548604	Pershing	Missouri	USA	R	1.0
PI 548520	Preston	Iowa	USA	R	1.3
PI 536636	Ripley	Ohio	USA	R	1.0
PI 536635	Sprite	Ohio	USA	R	1.0
PI 522236	Thomas	Georgia	USA	R	1.0
PI 548524	Weber	Iowa	USA	R	1.0
PI 508266	Young	North Carolina	USA	R	1.5
PI 548634	Zane	Ohio	USA	R	1.25
Old Japanese cultivars					
JP28882, PI 594233 A	Nourin 1	Ibaraki	Japan	S	4.0
PI495235	Nourin 2	Ibaraki	Japan	S	4.5
JP28883, PI 417200	Nourin 3	Ibaraki	Japan	S	3.5
JP27806, PI 594238	Nourin 4	Akita	Japan	S	2.0
JP27807	Nourin 5	Akita	Japan	S	—
JP27806	Tamamusume	Ibaraki	Japan	S	—
JP27808	Darumamasari	Akita	Japan	S	—
JP27809	Ugodaizu	Akita	Japan	S	—
JP29556, PI 592904	Hougyoku	Kumamoto	Japan	S	2.0
JP28491	Shinmejiro	Ibaraki	Japan	S	—
JP29552	Asomasari	Kumamoto	Japan	S	—
JP27810	Waseshiroge	Akita	Japan	S	—
JP29639, PI 423959	Asomusume	Kumamoto	Japan	S	2.0
JP27413	Hokkaihadaka	Hokkaido	Japan	S	—
JP29504	Koganedaizu	Saga	Japan	S	—
JP27406, PI 424199	Karikachi	Hokkaido	Japan	S	1.0
JP27811, PI 416913	Hatsukari	Akita	Japan	S	3.5
JP28492, PI 360848	Tachisuzunari	Ibaraki	Japan	S	2.5
JP29505, PI 417274	Sayohime	Saga	Japan	S	3.5
JP27412	Nagahajiro	Hokkaido	Japan	S	—
JP27407	Shinsei	Hokkaido	Japan	S	—
JP27436, PI 317335	Koganejiro	Hokkaido	Japan	S	1.5
JP27408	Tokachishiro	Hokkaido	Japan	R	—
JP27696, PI 342004	Nemashirazu	Akita	Japan	S	2.0
JP28493	Bonmimori	Ibaraki	Japan	S	—
JP28494, PI 360842	Okumejiro	Ibaraki	Japan	S	1.5
JP29506, PI 423987 A	Fujimusume	Saga	Japan	S	3.5
JP28861, PI 360847	Shiromeyutaka	Nagano	Japan	S	2.5
JP29559	Akisengoku	Kumamoto	Japan	S	—
Modern Japanese cultivars					
N/A (Nagano)	Tamadaikoku	Nagano	Japan	S	S
N/A (Hokkaido)	Iwaikuro	Hokkaido	Japan	S	S
N/A (Hokkaido)	Hayahikari	Hokkaido	Japan	R	R

Table S2. Cont.

Accession no.*	Accession name	Province	Country	*Pdh1*†	Shattering score‡
N/A (Tohoku)	Oosuzu	Akita	Japan	S	M
N/A (Nagano)	Tamamasari	Nagano	Japan	S	S
N/A (Nagano)	Suzukogane	Nagano	Japan	S	M
N/A (Tohoku)	Tamaurara	Akita	Japan	S	S
N/A (Tohoku)	Hatayutaka	Akita	Japan	S	M
N/A (Nagano)	Ayakogane	Nagano	Japan	S	M
N/A (Kyushu)	Erusuta	Kumamoto	Japan	S	S
N/A (Kyushu)	Sachiyutaka	Kumamoto	Japan	S	S
N/A (Tohoku)	Yumeminori	Akita	Japan	S	M
N/A (Hokkaido)	Yukihomare	Hokkaido	Japan	R	R
N/A (Nagano)	Suzukomachi	Nagano	Japan	R	R
N/A (Kyushu)	Kiyomidori	Kumamoto	Japan	S	R
N/A (Tohoku)	Fukuibuki	Akita	Japan	S	M
N/A (Tohoku)	Aomarukun	Akita	Japan	R	R
N/A (Hokkaido)	Yukishizuka	Hokkaido	Japan	S	M
N/A (Tohoku)	Suzusayaka	Akita	Japan	S	M
N/A (Nagano)	Tsubuhomare	Nagano	Japan	S	M
N/A (Tohoku)	Suzukaori	Nagano	Japan	S	M
N/A (Kyushu)	Kurodamaru	Kumamoto	Japan	S	R
N/A (Nagano)	Tsuyamohomare	Nagano	Japan	R	R
N/A (Tohoku)	Kinusayaka	Akita	Japan	S	M
N/A (Hokkaido)	Toyoharuka	Hokkaido	Japan	S	M
N/A (Kyushu)	Kotoyutaka	Kumamoto	Japan	S	S
N/A (Hokkaido)	Yukipirika	Hokkaido	Japan	S	S
N/A (Nagano)	Tachihomare	Nagano	Japan	S	M
N/A (Tohoku)	Suzuhonoka	Akita	Japan	S	M
Glycine soja					
PI 339871A	N/A	Cheju	Korea, South	S	—
PI 366120	N/A	Akita	Japan	S	—
PI 393551	N/A	Taiwan	Taiwan	S	—
PI 407027	N/A	Akita	Japan	S	—
PI 407131	N/A	Kumamoto	Japan	S	—
PI 407140	N/A	Kumamoto	Japan	S	—
PI 407170	N/A	Kyonggi	Korea, South	S	—
PI 407275	N/A	Kyonggi	Korea, South	S	—
PI 407282	N/A	Cheju	Korea, South	S	—
PI 407288	N/A	Jilin	China	S	—
PI 407301	N/A	Jiangsu	China	S	—
PI 447004	N/A	Jilin	China	S	—
PI 458536	N/A	Heilongjiang	China	S	—
PI 458538	N/A	Heilongjiang	China	S	—
PI 464935	N/A	Jiangsu	China	S	—
PI 468400A	N/A	Ningxia	China	S	—
PI 483464A	N/A	Ningxia	China	S	—
PI 483465	N/A	Shaanxi	China	S	—
PI 518282	N/A	Unknown	Taiwan	S	—
PI 549046	N/A	Shaanxi	China	S	—
PI 562559	N/A	Cholla Puk	Korea, South	S	—
PI 562565	N/A	Cholla Puk	Korea, South	S	—
PI 597459D	N/A	Shandong	China	S	—
PI 326582A	N/A	Primorye	Russia	S	—
PI 468916	N/A	Liaoning	China	S	—
Others					
PI 548573	Harosoy	Ontario	Canada	R	—
JP27423, PI 360850	Wasekogane	Hokkaido	Japan	R	1.0
JP86520, PI 593971	Kariyutaka	Hokkaido	Japan	R	2.0
N/A (Hokkaido)	Tokei 992	Hokkaido	Japan	R	
PI 597482	Sinpaldalkong	Suweon	Korea, South	R	1.0
PI 540556	Jack	Illinois	USA	R	1.0
JP27541, PI 594301	Toyomusume	Hokkaido	Japan	S	4.0
JP28862, PI 385942	Enrei	Nagano	Japan	S	2.5
N/A (Hokkaido)	Shokukei 32	Hokkaido	Japan	S	

Table S2. Cont.

Accession no.*	Accession name	Province	Country	*Pdh1*[†]	Shattering score[‡]
JP67666, PI 561396	Tachinagaha	Nagano	Japan	S	3.5
PI 416937	Houjaku Kuwazu	Kanto and Tosan	Japan	S	3.0
JP29668, PI 506675	Fukuyutaka	Kumamoto	Japan	S	2.0
JP68385, PI 561395	Suzuyutaka	Akita	Japan	S	4.5
PI 594021	Keunolkong	Yeongnam	Korea, South	S	—
JP28856, PI 507045	Misuzudaizu	Nagano	Japan	S	3.0

N/A, not available; —, none.

*Accessions whose numbers are prefixed as indicated were kindly provided by the following agencies: PI or FC, the Agricultural Research Service of the USDA; JP, NIAS, Japan; N/A (Nagano), Nagano Vegetable and Ornamental Crops Experiment Station, Japan; N/A (Hokkaido), Agricultural Research Department of Hokkaido Research Organization, Japan; N/A (Tohoku) or N/A (Kyushu), NARO.

[†]"S" and "R" indicate shattering-susceptible and shattering-resistant genotypes at the SNP of *Pdh1*.

[‡]Shattering scores of accessions with PI numbers indicate the "SHATLATE" values published by the Germplasm Resources Information Network, USDA (www.ars-grin.gov/). Scores are based on the percentage of open pods 2 wk after harvest, as follows: 1, no shattering; 2, 1–10%; 3, 10–25%; 4, 25–50%; 5, >50%. For Japanese modern cultivars, the degrees of shattering resistance are shown as follows (based on the data registered with the Ministry of Agriculture, Forestry, and Fisheries, Japan): "S", shattering-susceptible; "M", moderate shattering; "R", shattering-resistant.

Table S3. Primers used in this study

Purpose	ID	Direction	5′-primer sequence-3′	Remarks
Mapping	Sca184_401k	F	TGCTCCATGTGCTATATAATTGCT	Position:29547k, Indel
		R	AAGCTCGTTCTTGTTGATGG	
	SRM0	F	GCCAGCCTTGTCTGTCATTT	Position:29573k, Indel
		R	TGATGATCAATGGTCAGATTCA	
	scrf2_gap	F	CCCTACAATTACCCCACCAA	Position:29595k, Indel
		R	GCACGGATAAGCTCTTCGAT	
	scrf2_SNP1	F	CCCTACAATTACCCCACCAA	Position:29596k, SNP
		R	TCTGACTTCCACAGCAAAATATTCCACACCA	
	Gm16_29608k_SNP	F	TTCTAGAAACGTGGCAATCG	Position:29608k, SNP
		R	CCATCTTCTACTTGAAATTTGGCTA	
	Gm16_29616k_ID	F	CAACCCATTCAATTAGGCTCA	Position:29616k, Indel
		R	TCAACGAAGTAAAGTTTTAACACCA	
	Gm16_29622k_ID	F	TGATGCTCTTGTAACTTGAGATGATG	Position:29622k, Indel
		R	ATAACTCAACGGCTCAGCTCCTCAA	
	SRM1	F	AGAGCAAGAAATCACGTTGCA	Position:29634k, Indel
		R	CACCTCACCCCTTTTTCTCA	
	Sca184_281k (SRM2)	F	AATCGTATTAAAATTGAAGGCATGT	Position:29667k, Indel
		R	AGGGGTTGAGGATGAGGAGT	
	Sca184_267k	F	GGTGTTTGGCTCGTTAATGG	Position:29681k, Indel
		R	TCACCTACATGCATGCAACC	
Genomic cloning	Glyma16g25570.1	F	TCTGACTTCCACAGCAAAATATTCCACACCA	
		R	AGGCAAGCAAGTTTAGGGCATCTTCACGTA	
	Glyma16g25580.1 (*Pdh1*)	F	GGCTCTAAAAATGCATCAATCACTGGTCCAA	
		R	CAACAAAACCACCGTCACCCATAAATGAGA	
RT-PCR	Glyma16g25570.1	F	GAGGGAGGCGTTTTACGAC	ORF1
		R	GACGTGGCAACCATGACTC	
	Glyma16g25580.1 (*Pdh1*)	F	CGAAGCCGGAAATAAACTTG	ORF2
		R	ACAGCACTGATCATCGTGGA	
	CYP2	F	CGGGACCAGTGTGCTTCTTCA	
		R	CCCCTCCACTACAAAGGCTCG	
5′ RACE	DIR5RACE1st	F	—	Reverse transcription
		R	GTTAAGTTTGCACCTTCT	
	DIR5RACE2nd	F	GGCCACGCGTCGACTAGTACGGGIIGGGIIGGGIIG	
		R	TAAGTGCTTGCATGGTGCAT	
	DIR5RACE3rd	F	GGCCACGCGTCGACTAGTAC	
		R	AAGAACACAACGAGGGCAAC	
3′ RACE	Oligo(dT) adapter	F	—	Reverse transcription
		R	GGCCACGCGTCGACTAGTACTTTTTTTTTTTTTTTT	
	DIR3RACE2nd	F	GCCCTCGTTGTGTTCTTCAT	
		R	GGCCACGCGTCGACTAGTAC	
	DIR3RACE3rd	F	GGTAGGGCACAAGGCTCTTA	
		R	GGCCACGCGTCGACTAGTAC	
In situ hybridization	In situ probe	F	AGTCCTTTCCTATTTCAAAGTCCTCT	
		R	TTCTTGGTGTCGTAAATGTAAGAGCCT	
*Pdh1*SNP	Dir_CAPS	F	GCCCTCGTTGTGTTCTTCAT	SNP
		R	GCGTTGCTTCCGTTGTAGAT	
	Dir_SNP_ARMS	F	CTCTTGGCATAGCTAGGGAAAAGCACTA	
		R	GAAAACCACTATGTTTCCGAAGTGGAAC	
		F	GAAGAGGCCACAACATGCACCATACT	
		R	TGTCGTGGAAGAAGAGGACTAAGTGTTT	
Pdh1 sequencing	Pdh1seq	F	GGCATAGCTAGGGAAAAGCAC	Table S1
		R	CCATTTATTTTTAGTAAACAAACTTTTCATG	
Transformation	DirigentG	F	TGAACAAAAATCTCACGGATTACATCAGCA	
		R	GAGCTCAATATGGAAATTAAGTCCGAACCAGACTAGTCACC	Underlined: addition of a *Sac*I site

—, none.