Theor Appl Genet (2008) 117:479–487
DOI 10.1007/s00122-008-0792-5

ORIGINAL PAPER

QTL analysis of cleistogamy in soybean

Nisar A. Khan · Stephen M. Githiri · Eduardo R. Benitez · Jun Abe · Shinji Kawasaki · Takeshi Hayashi · Ryoji Takahashi

Received: 12 January 2008 / Accepted: 2 May 2008 / Published online: 27 May 2008

Abstract Early-maturing cultivars of soybean [*Glycine max* (L.) Merr.] native to the shores of the Sea of Okhotsk (Sakhalin and Kuril Islands) and eastern Hokkaido (northern Japan) have a strong tendency to produce cleistogamous flowers throughout their blooming period. A previous study revealed that cleistogamy is controlled by a minimum of two genes with epistatic interaction, one of which is associated with a maturity gene responsible for insensitivity to incandescent long daylength (ILD). This study was conducted to determine the genetic basis of cleistogamy in more detail by QTL mapping. F_2 to F_4 progenies derived from a cross between a cleistogamous cv. Karafuto-1 and a chasmogamous cv. Toyosuzu were used. A molecular linkage map spanning 2,180 cM comprising 500 markers was constructed using 89 F_2 plants. The markers were distributed in 25 linkage groups. An interval mapping method to analyze categorical traits identified four QTLs for cleistogamy, cl1, cl2, cl3 and cl4, in molecular linkage groups (MLGs) C2, D1a, I and L, respectively. Alleles derived from Karafuto-1 had additive effects to increase probability of cleistogamy at cl3 and cl4, whereas the alleles had additive effects to decrease the probablity at cl1 and cl2. Progeny test confirmed the effects of cl3, which had the highest LOD score (5.20). Composite interval mapping revealed four QTLs for flowering date, fd5–fd8. Judging from relative location with markers and association with ILD responses, fd7 and fd8 may correspond to maturity genes *E4* and *E3*, respectively. cl3 and cl4 were located at similar positions as fd7 and fd8, suggesting that the two maturity genes may control cleistogamy by either pleiotropy or close linkage.

Communicated by F. J. Muehlbauer.

N. A. Khan · R. Takahashi
University of Tsukuba, 2-1-18 Kannondai,
Tsukuba, Ibaraki 305-8518, Japan

E. R. Benitez · R. Takahashi (✉)
National Institute of Crop Science,
2-1-18 Kannondai, Tsukuba, Ibaraki 305-8518, Japan
e-mail: masako@affrc.go.jp

J. Abe
Research Faculty of Agriculture,
Hokkaido University, Sapporo, Hokkaido 060-8589, Japan

S. Kawasaki · T. Hayashi
National Institute of Agrobiological Sciences,
2-1-2 Kannondai, Tsukuba, Ibaraki 305-8602, Japan

Present Address:
S. M. Githiri
Department of Crop Science,
University of Nairobi, P.O. Box 30197, Nairobi, Kenya

Introduction

Cleistogamy, or the production of open (chasmogamous, CH) and closed (cleistogamous, CL) floral forms by one species, is widespread among the angiosperms. Culley and Klooster (2007) noted 693 species, distributed over 228 genera and 50 families for cleistogamy. They classified cleistogamy into three categories: dimorphic cleistogamy in which prominent floral differences in CL and CH floral morphology result from divergent developmental pathways; complete cleistogamy in which individual plants produce only CL flowers; and, induced cleistogamy in which the environment arrests the development of CH flowers prior to anthesis and results in a mechanical failure of the flowers to open, resulting in the production of a CL flower.

Cleistogamy has been observed in soybean [*Glycine max* (L.) Merr.] (Takahashi et al. 2001). Soybean usually produces both CH and CL flowers on an individual plant; fertilization occurs without opening of petals in CL flowers. Hence, based on the classification of Culley and Klooster (2007), the flower dimorphism of soybean is classified as induced cleistogamy.

Cleistogamy may be advantageous under severe environmental conditions because the energetic costs (i.e., sepals, petals and nectar) of CL flowers appears to be considerably lower than that of CH flowers. Charles Darwin (1897) speculated that cleistogamy may be advantageous because CL flowers produce an abundant supply of seeds with less expenditure than CH flowers. Shemske (1978) evaluated the energetic costs of CH and CL flowers in *Impatiens pallida* and found that CH flowers had an energetic investment more than 100 times higher than that of CL flowers. Further, CL flowers possibly shelter pollen from chilling temperatures at fertilization, because chilling temperatures hinder pollen formation, anther dehiscence and fertilization in soybean (Goto and Yamamoto 1972).

Early-maturing landraces (maturity group 000–00) native to the shores of the Sea of Okhotsk (Sakhalin and Kuril Islands) and eastern Hokkaido (northern Japan) usually produce only CL flowers when cultivated in Hokkaido. In these cultivars, fertilization occurs without opening of petals, or without any appearance of petals at anthesis. However, they have been observed to produce CH flowers at the early flowering stage during years with high temperatures or when cultivated in the southern regions.

The early-maturing CL landraces have been used in breeding for chilling tolerance in Japan and Sweden (Holmberg 1973). Low temperatures at the flowering stage, which is most sensitive to chilling stress, induce flower and pod abortion (Holmberg 1973; Hume and Jackson 1981; Takahashi and Asanuma 1996). Minimum temperatures required for good flowering and pod set vary among cultivars (Holmberg 1973; Hume and Jackson 1981). The early maturing landraces and some of their descendants such as the cultivar Fiskeby V have somewhat lower critical temperatures, and flowering and seed formation are generally not interrupted by low temperatures (Holmberg 1973; Hume and Jackson 1981). Soybean breeders in Hokkaido have presumed that cleistogamy might be associated with chilling tolerance in terms of seed yield reduction (Dr. T. Narikawa, personal communication, 1988).

Takahashi et al. (2001) conducted genetic analysis of cleistogamy using F_1 plants, F_2 population and F_3 families produced by crossing between Karafuto-1 (early-maturing cultivar introduced from Sakhalin) and a CH cultivar, Toyosuzu. F_1 plants had CH flowers, indicating that chasmogamy was dominant over cleistogamy. Analysis of F_2 population and F_3 families generated segregation data that was close to a two-gene model with epistatic interactions, although a portion of the pooled F_3 data on the frequency of CH segregants from CL families significantly deviated from the model. The results suggested that a minimum of two genes with epistatic effects were involved in the genetic control of cleistogamy. Furthermore, cleistogamy was associated with early flowering in the F_2 and F_3 populations. A gene for cleistogamy was linked to one of the recessive genes responsible for ILD response. Based on the segregation of ILD response of F_3 families, Takahashi et al. (2001) suggested a two-gene model in which two recessive genes were involved in ILD insensitivity of Karafuto-1. The lack of association between ILD response and pubescence color gene *T* that is linked with a maturity gene *E1* indicates that *E1* may not be a maturity gene associated with cleistogamy (Takahashi et al. 2001). The insufficient availability of genetic markers hampered detailed genetic analysis of cleistogamy and ILD response in the previous study. This study was conducted to further investigate the genetic basis of cleistogamy using DNA markers.

Materials and methods

Plant materials

A CL cultivar, Karafuto-1, and a CH cultivar, Toyosuzu, were used in this study. Karafuto-1 is a pure-line selected from an early-maturing landrace introduced from Sakhalin (maturity group 00); it has tawny pubescence and brown hilum (*i-ii-irrTT*) and is insensitive to ILD. Karafuto-1 produces CL flowers throughout its flowering period except during years with high temperatures (Fig. 1). Toyosuzu is a cultivar (maturity group II) developed at the Tokachi Agricultural Experiment Station by crossing Gedenshirazu-1 and Toshidai-7910 (a landrace from Sakhalin). Toyosuzu has gray pubescence and yellow hilum (*IIrrtt*) and is sensitive to ILD. In Hokkaido, Toyosuzu usually produces CH flowers irrespective of environmental conditions except during the later stages of flowering. Karafuto-1 was crossed with Toyosuzu in 1997 and 2003. A total of 98 F_2 plants derived from the cross in 1997 were cultivated and cleistogamy was evaluated at the Tokachi Agric. Exp. Stn., Memuro, Hokkaido, Japan (42°53'N, 143 ° 05'E) in 1998 as described in Takahashi et al. (2001). Date of anthesis was recorded for each F_2 plant for QTL analysis. F_3 families were cultivated at Hokkaido University (43°03'N, 141°20'E) in 1999 to evaluate ILD-response as described in Takahashi et al. (2001).

To ascertain the reproducibility of QTLs, three F_4 families segregating for cleistogamy in a 3:1 ratio were generated from the cross of 2003. Briefly, F_3 families derived

Fig. 1 Chasmogamous flower of Toyosuzu (*left*) and cleistogamous flower of Karafuto-1 (*right*) at fertilization

from six CH plants selected from the F_2 population ($n = 67$) were evaluated for segregation of cleistogamy at Hokkaido University in 2005. Among them, two F_3 families segregating in the 3:1 ratio (#46 and #58) were chosen to generate F_4 segregating families. Three F_4 families (#46-1, #46-14 and #58-2) with monogenic segregation were used for the association test between SSR marker genotype and cleistogamy. From each of the F_4 families, 21 to 27 plants were grown at the Hokkaido University in 2006.

Evaluation of cleistogamy and ILD response

Varietal differences in floral forms were evident at an early period of flowering, so cleistogamy of each plant was determined by evaluating the flowers that fertilized on the first date of anthesis (fertilization). Date of anthesis in CL plants was visually evaluated from the size of the flower buds, and it was confirmed by comparing the sizes of developing pods 5 days after anthesis. For QTL analysis, individual F_2 plants were classified based on their floral morphology as follows: CH, open flowers with fully expanded petals; or CL, flowers with slightly expanded petals that protruded out of calyxes or flowers whose petals did not emerge from the calyxes. ILD response was evaluated in the F_3 families as described in Takahashi et al. (2001). Individual plants were classified into the following three types: Karafuto-1 type, Toyosuzu type and intermediate type whose growth stage reached R2 to R3 (Fehr et al. 1971). F_3 families were categorized into ILD-insensitive families, ILD-sensitive families, and heterozygous families. ILD-insensitivity of F_2 plants was estimated from that of respective F_3 families and was used for QTL analysis.

Genotyping of morphological traits, isozyme and SSR markers

Genotypes of *T* gene for pubescence color were visually determined in the F_3 families. Assay of three isozyme loci, *Mpi* (mannose-6-phoshate isomerase mobility variant), *Idh2* (isocitrate dehydrogenase mobility variant) and *Pgm1* (phosphoglucomutase mobility variant) followed the method described by Abe et al. (1992). Total DNA of the parents and the F_2 plants was extracted from trifoliolate leaves by the CTAB method (Murray and Thompson 1980). Total DNA was extracted from a bulk of five F_5 seeds, each from plants of F_4 families segregating for cleistogamy to evaluate genotypes of F_4 plants according to Kamiya and Kiguchi (2003). A total of 1,147 pairs of SSR primers developed by USDA (Cregan et al., 1999; Song et al., 2004) and Chiba University (Dr. K. Harada, personal communication) were used to screen the parents. The PCR mixture for SSR analysis contained 30 ng of genomic DNA, 5 pmol of primer, 10 pmol of nucleotides, and 1 unit of ExTaq enzyme in 1 x ExTaq buffer supplied by the manufacturer (Takara Bio, Ohtsu, Japan) in a total volume of 10 µl. The initial 12 min denaturation at 94°C was followed by 40 cycles of 30 s denaturation at 94°C, 30 s annealing at 50°C, and 1 min extension at 68°C. A final 5 min extension at 68°C completed the program. The PCR was performed in an Applied Biosystems 9700 thermal cycler (Applied Biosystems, Foster City, CA, USA). The PCR products were separated on 8% nondenaturing acrylamide gels, and the fragments were visualized by staining with ethidium bromide.

AFLP analysis

AFLP analysis was performed using a total of 1,536 primer pairs according to Kawaguchi et al. (2001). PCR reactions were performed with *Eco*RI- and *Mse*I-digested DNA ligated to two sets of primers using ExTaq. PCR products were separated on 13% nondenaturing acrylamide gels (38: 2). The fragments were visualized using vistra green according to the manufacturer's instructions (GE Healthcare Bio-Science, Piscataway, USA). Designation of AFLP markers followed the report of Mano et al. (2001).

Statistical analysis

The markers were tested by Chi-square analyses for segregation in a 1:2:1 or 3:1 ratio. A linkage map was constructed using MAPMAKER/EXP version 3.0 (Lander et al. 1987) with the threshold LOD score of 3.0. QTL analysis of flowering date was performed by composite interval mapping (Zeng 1993) using the QTL Cartographer ver. 2.0 (Basten et al. 2001) with the threshold LOD value of 2.0.

Because the cleistogamy phenotypes of plants were recorded with the binary traits, CL and CH, an interval mapping method developed by Hayashi and Awata (2006) for analyzing categorical traits was applied, where logistic regression was considered with QTL genotypes used as covariates for modeling the probabilities of plants being classified into CL or CH. The threshold value of LOD score for the analysis of cleistogamy was investigated by permutation test with 5,000 repetitions and determined as 2.0 corresponding to genome-wide 1% significance. QTL analysis of cleistogamy was thus performed with the threshold LOD value of 2.0. QTL analysis of ILD response was carried out by nonparametric analysis (Kruskal–Wallis analysis) using the MapQTL 4.0 (Van Ooijen et al. 2002).

Results

Cleistogamy, ILD response and flowering date

Cleistogamy and ILD response of the F_2 population are presented in Table 1. The F_2 plants were segregated into 77 CH and 21 CL plants. ILD response of the F_2 plants was estimated from the segregation of F_3 families. Of the 98 F_3 families, 88 families with a minimum of 15 plants each were classified into three classes, viz., families fixed for ILD-sensitivity, families fixed for ILD-insensitivity and families segregating for ILD response. A total of 44 F_2 plants were classified as ILD-sensitive, 8 plants as ILD-insensitive and the others as heterozygous. All ILD-insensitive plants had CL flowers. Karafuto-1 flowered 3.5 days earlier than Toyosuzu (July 23). The F_2 population started to flower between July 15 and 25.

Polymorphism and linkage

Out of 98 F_2 plants that grew normally in 1998, 9 plants were excluded from QTL analysis because of DNA impurity. A total of 522 markers including 1 morphological, 3 isozyme, 195 SSR, and 323 AFLP markers were used for linkage mapping. Among the 522 markers, 500 markers comprised 25 linkage groups containing 20 known linkage groups (Cregan et al. 1999; Song et al. 2004). The remaining 22 markers including *T* remained unlinked, although it is known that *T* is on MLG C2. On average, 20 markers were placed in each linkage group. The total map coverage excluding unlinked markers was 2180 cM with an average of 4.4 cM between loci. MLG J contained only three markers covering 14.8 cM.

QTL analysis of cleistogamy, flowering date and ILD response

Interval mapping revealed four significant QTLs for cleistogamy at a genome-wide 1% level of significance. These QTLs, referred to as cl1, cl2, cl3 and cl4 were located on MLGs C2, D1a, I and L, respectively (Table 2; Fig. 1). The LOD score of cl3 was highest (5.20). The allele derived from Karafuto-1 has an effect increasing the probability of plant being classified into CL at cl3 and cl4 in comparison with that from Toyosuzu, whereas the allele from Karafuto-1 showed an effect decreasing the probability of CL at cl1 and cl2, as shown in Table 2.

Composite interval mapping revealed four QTLs for flowering date, fd5 in MLG D1a, fd6 in MLG D1b, fd7 in MLG I and fd8 in MLG L (Table 3; Fig. 1). LOD scores of fd7 (5.84) and fd8 (6.22) were high and they explained 19.6 and 18.8% of the total variance, respectively. Additive effects indicated that the Karafuto-1 genotype of markers around the four QTLs reduced the number of days to flowering. Kruskal–Wallis analysis revealed that markers around fd7 and fd8 were significantly associated with ILD response (Fig. 2). The cl3 and fd7, and cl4 and fd8 were overlapped in the MLG I and L, respectively (Fig. 2).

To further investigate the association between cleistogamy and flowering date, LOD scores for cleistogamy and flowering date were plotted around the QTLs detected in this study (Fig. 3). The LOD score plots indicated that cl3 and fd7, and cl4 and fd8 were located in similar positions. In contrast, cl1 and cl2 exhibited no evident association with days to flowering.

The effect of cl3, a QTL with the highest LOD score, was confirmed in the F_4 progeny data using the proximate SSR marker Sat_219. Table 4 shows the data of the three

Table 1 Association between cleistogamy and ILD-response in an F_2 population developed by a cross between soybean cultivars, Karafuto-1 and Toyosuzu

Number of plants					
Cleistogamy	ILD-sensitive	Heterozygous	ILD-insensitive	Not determined[a]	Total
CH	42	29	0	6	77
CL	2	7	8	4	21

ILD response of F_2 plants was estimated from ILD response of F_3 families

[a] ILD response of F_3 families could not be determined

Table 2 QTLs responsible for cleistogamy phenotypes (cl1 to cl4) observed in an F_2 population developed by a cross between soybean cultivars, Karafuto-1 and Toyosuzu

QTL name	Linkage group	Proximal marker	Position[a] (cM)	LOD score	Probability of CL[b]		
					QQ	Qq	qq
cl1	C2	Satt640	26.8	2.13	0.000	0.286	0.219
cl2	D1a	e21m36-5-2	64.1	2.14	0.047	0.342	0.106
cl3	I	Sat 219	43.2	5.20	0.529	0.109	0.000
cl4	L	Satt 229	45.8	3.82	0.571	0.119	0.038

QTL analysis was carried out by the method of Hayashi and Awata(2006)

[a] Distance from the top of each linkage group obtained in this study

[b] Predicted probability of plants being classified into cleistogamy given the QTL genotypes, QQ, Qq and qq, where Q and q indicate alternative QTL alleles derived from Karafuto-1 and Toyosuzu, respectively. The probability was calculated as described in Hayashi and Awata (2006)

Table 3 QTLs responsible for flowering date (fd5 to fd8) observed in an F_2 population developed by a cross between soybean cultivars, Karafuto-1 and Toyosuzu

QTL name	Linkage group	Proximal marker	Position[a] (cM)	LOD score	Additive effect[b]	Dominance effect	Variance explained (%)
fd5	D1a	Satt531	8.0	2.30	−0.56	−0.91	6.6
fd6	D1b	Satt296	11.7	2.98	−0.80	−0.70	7.4
fd7	I	e9m26-6	53.0	5.84	−1.35	0.42	19.6
fd8	L	Satt229	42.3	6.22	−1.40	−0.12	18.8

QTL analysis was conducted by the usual composite interval mapping

[a] Distance from the top of each linkage group obtained in this study

[b] Additive effects of each QTL are those of Karafuto-1 allele in contrast to Toyosuzu allele

families regarding segregation of cleistogamy and genotypes at Sat_219. In total, 96% of CH F_4 plants had the Toyosuzu-type or heterozygote genotype, whereas CL F_4 plants exclusively had the Karafuto-1 or heterozygote genotype at Sat_219. The results confirmed that cl3 is reproducible across generations.

Discussion

Low map coverage of the F_2 population could be explained by the relatedness of the parents. Toshidai-7910 (a parent of Toyosuzu) and Karafuto-1 are both landraces from Sakhalin and belong to the Namikawa-group having basically similar phenotypes including maturity, morphology and cleistogamy (Japan Beans and Peas Foundation 1991). Considering the large number of markers, we had expected that most of the QTLs in the polymorphic regions between the parents would be covered in this study.

Four QTLs for cleistogamy, cl1 to cl4, were detected. Alleles derived from Karafuto-1 increased the probability of CL plants at cl3 and cl4, whereas cl1 and cl2 had cleistogamy enhancing alleles coming from Toyosuzu. The results confirmed the complex inheritance of cleistogamy in our previous report (Takahashi et al., 2001). The two major QTLs, cl3 and cl4, were located at similar positions to QTLs for flowering date, fd7 and fd8 (Fig. 1).

Eight loci have been reported to control time to flowering and maturity in soybean: *E1*, *E2* (Bernard 1971), *E3* (Buzzell 1971), *E4* (Buzzell and Voldeng 1980), *E5* (McBlain and Bernard 1987), *E7* (Cober and Voldeng 2001), and *E6* (Bonato and Vello 1999) and *J* (Ray et al. 1995) for long juvenility. Dominant alleles at *E1* to *E5* and *E7* delay time to flowering and maturity. The *E3*, *E4* and *E7* loci are involved in the initiation of flowering under ILD. The recessive alleles, *e3* and *e4* jointly confer insensitivity to ILD (Buzzell 1971; Buzzell and Voldeng 1980). When combined with *e3* and *e4*, *E1* markedly retards flowering under ILD or natural daylength relative to *e1* (Cober et al. 1996). *E1* and *E7*, *E2*, *E3* and *E4* loci were determined to be located on MLG C2, O, L and I, respectively (Cregan et al. 1999; Cober and Voldeng 2001; Abe et al. 2003; Molnar et al. 2003; Matsumura et al. 2008).

Abe et al. (2003) mapped *E4* on MLG I with the order of Satt239–Satt496–*E4*–*Enp*–Satt354. The present results indicate that the largest QTL for flowering date lies close to Sat_219 and AB002807. Sat_219 and AB002807 are located between Satt496 and *Enp* in the comprehensive genetic map (Song et al. 2004) suggesting that fd7 corresponds to *E4*. Further, fd8 may correspond to *E3*, because

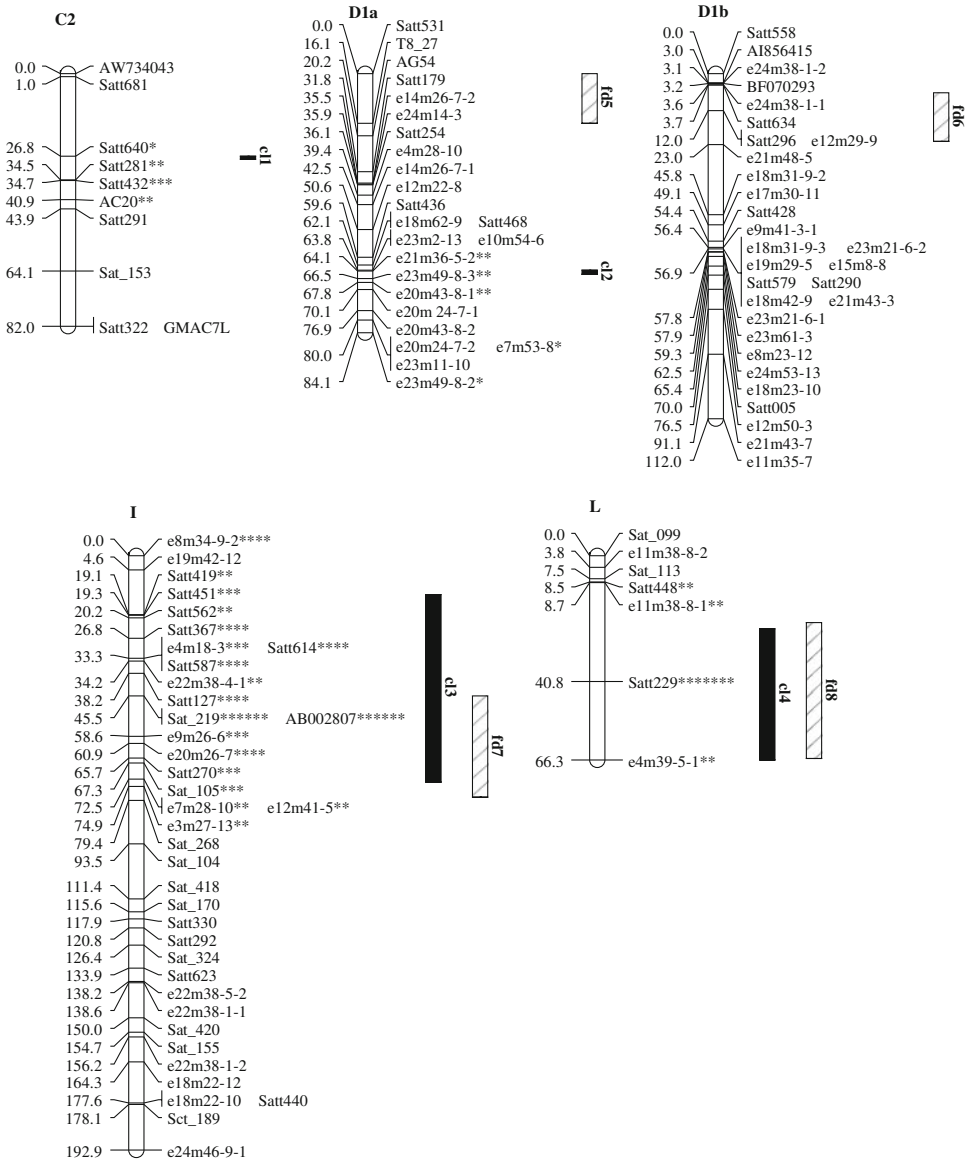

Fig. 2 QTLs for cleistogamy (*black bars*) and flowering date (*hatched bars*). Four QTLs for cleistogamy (cl1 to cl4) and four QTLs for flowering date (fd5 to fd8) were found. The name of each linkage group is indicated at the *top*. The linkage groups were named using the consensus map of Cregan et al. (1999). Distances of markers from the top of each linkage group are shown on the *left*. SSR and AFLP markers are shown in *normal font* (AFLP markers start with *e*). Markers significantly associated with ILD response are shown by *asterisks* (*, **, ***, ****, *****, ******, and ******* represent significance level of 5, 1, 0.5, 0.1, 0.05, 0.01 and 0.005% by Kruskal–Wallis test, respectively). The cl3 and fd7, and cl4 and fd8 were overlapped in MLGs I and L, respectively

molecular linkage mapping revealed that *E3* locates in the vicinity of Satt229 in MLG L (Molnar et al. 2003; Watanabe et al. 2004). The existence of QTLs for ILD response around the two regions supports this conclusion.

Based on the mapping positions and association with ILD response, Karafuto-1 and Toyosuzu may have the *e3e4* and *E3E4* genotypes, respectively. The estimated genotype for Toyosuzu is consistent with our unpublished observation that Toyosuzu displayed delayed flowering under ILD and fluorescent long daylength conditions. The pubescence color gene *T*, a classical tagging marker for *E1*, was not associated with cleistogamy or ILD response in this study. Therefore, the maturity gene associated with cleistogamy is not *E1*, as presumed by Takahashi et al. (2001). The present results further confirmed the proposal of Takahashi et al. (2001) that two recessive alleles of ILD-responsive genes might be involved in ILD-insensitivity of Karafuto-1. This is consistent with the two-gene model (*e3e3 e4e4*) for ILD insensitivity proposed by Saindon et al. (1989).

Soybean cultivated at high latitudes and altitudes frequently suffer from low temperatures. Chilling stress retards growth, causes abortion of flowers and immature pods and reduces the final seed yield. Furthermore, chilling temperatures (about 15°C) during flowering induce browning and cracking of seed coats (Sunada and Ito 1982). Takahashi and Abe (1999), and Benitez et al. (2004) treated soybean cv. Harosoy (*e1e2E3E4e5E7*) and its near-isogenic lines (NILs) for *E1* to *E5* and *E7* with chilling temperatures. Among the maturity genes, dominant alleles *E1* and *E5* were most effective in suppressing both pigmentation and cracking. Further, *E4* exhibited a unique response; the recessive allele suppressed the low-temperature induced seed coat deterioration, in contrast to the other loci whose dominant alleles suppressed the deterioration.

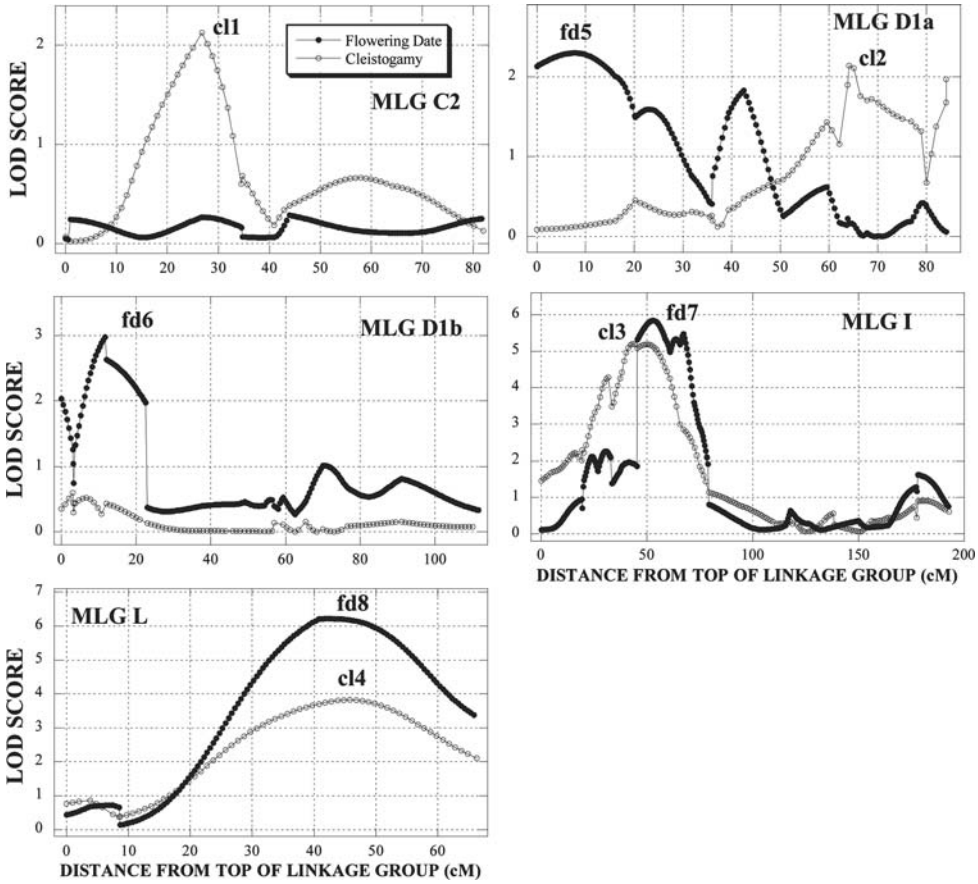

Fig. 3 The LOD score plots of cleistogamy and flowering date around the QTLs for cleistogamy or flowering date in MLG C2 (cl1), MLG D1a (fd5 and cl2), MLG D1b (fd6), MLG I (cl3 and fd7), and MLG L (cl4 and fd8). LOD score of cleistogamy was calculated by an interval mapping method for categorical traits according to Hayashi and Awata (2006). LOD score of flowering date was calculated by composite interval mapping. LOD score plots for cleistogamy and flowering date overlapped around cl3 and fd7 in MLG I, and cl4 and fd8 in MLG L

Table 4 Association between genotype at Sat_219 around the peak of cl3 and cleistogamy in three soybean F_4 families (#46-1, #46-14 and #58-2) segregating for cleistogamy

	Cleistogamous			Chasmogamous		
Family	Karafuto-type	Heterozygous	Toyosuzu-type	Karafuto-type	Heterozygous	Toyosuzu-type
#46-1	5	1	0	0	14	3
#46-14	7	0	0	0	13	6
#58-2	5	1	0	2	7	6
Total	17	2	0	2	34	15

Number of plants having respective genotypes at Sat_219 is shown

Takahashi et al. (2005) treated Harosoy and its NILs for *E1e3e4* with chilling temperatures at flowering. Weight of seed per plant in NILs with *E1e3e4* was higher than NILs with *e1E3E4* under chilling treatments, suggesting that the allelic combination of *E1e3e4* is preferable to *e1E3E4* in increasing seed yield under chilling conditions. Higher seed yield of NIL with *E1e3e4* was attained not from seed size but from increase of pod number. Thus, the recessive allele of the *E4* locus may be useful in breeding for chilling tolerance in terms of both yield and quality of seeds.

The *e4* allele has been found in early-maturing landraces native to the shores of the Sea of Okhotsk and in Swedish breeding lines (Abe et al. 2003). Chilling tolerance of a Swedish cultivar Fiskeby V is presumed to be derived from a Sakhalin landrace, Namikawa, which has a phenotype similar to Karafuto-1 (Holmberg 1973). Hume and Jackson (1981) evaluated pod-forming ability under chilling temperatures using 36 soybean cultivars. In all cases where pedigree could be determined, the most chilling tolerant cultivars were related to Fiskeby V.

The present study revealed that *E4* may control cleistogamy either due to pleiotropy or close linkage. The existence of small effects on cleistogamy by maturity gene *E3* suggests that pleiotropic effects might be responsible. Maturity genes might affect cleistogamy through proteins located downstream of the gene products in the signal transduction

pathway leading to a transition from vegetative to reproductive growth. Genes directly responsible for cleistogamy should be investigated by molecular genetic studies including microarray or subtraction experiments. Alternatively, gene(s) for cleistogamy might exist in the close vicinity of *E4*. In both cases (pleiotropy and close linkage), a short chromosomal segment harboring gene(s) for cleistogamy and chilling tolerance (*e4*) exist in MLG I. Cleistogamy may have acted as an indicator of the chromosomal segment and may have led soybean breeders in Hokkaido to associate cleistogamy with chilling tolerance. However, it is still uncertain whether cleistogamy is directly related to chilling tolerance. Molecular genetic analysis of the maturity genes and their signal transduction pathways may provide further insight into the mechanism of low-temperature response and/or cleistogamy in soybean. A comprehensive survey of the chromosomal segment around *E4* might reveal a genomic region where genes responsible for adaptation to high latitude regions with low temperatures, long daylengths and short growing seasons are clustered.

Acknowledgments The authors thank Dr. T. Narikawa and Dr. K. Harada (National Institute of Agrobiological Sciences) for providing information, and Dr. Joseph G. Dubouzet (National Institute of Agrobiological Sciences) for critical reading of the manuscript. This study was partially supported by grants-in-aid from the Ministry of Agriculture, Forestry and Fisheries, Japan, the Postdoctoral Fellowship for Foreign Researchers from Japan Society for the Promotion of Science (JSPS) to Githiri and Benitez, and the Japanese government (MEXT) scholarship to Khan.

References

Abe J, Ohara M, Shimamoto Y (1992) New electrophoretic mobility variants observed in wild soybean (*Glycine soja*) distributed in Japan and Korea. Soyb Genet Newsl 19:63–72

Abe J, Xu D, Miyano A, Komatsu K, Kanazawa A, Shimamoto Y (2003) Photoperiod-insensitive Japanese soybean landraces differ at two maturity loci. Crop Sci 43:1300–1304

Basten CJ, Weir BS, Zeng ZB (2001) QTL Cartographer, Version 2.0. Department of Statistics. North Carolina State University, Raleigh, USA

Benitez ER, Funatsuki H, Kaneko Y, Matsuzawa Y, Bang SW, Takahashi R (2004) Soybean maturity gene effects on seed coat pigmentation and cracking in response to low temperatures. Crop Sci. 44:2038–2042

Bernard RL (1971) Two major genes for time of flowering in soybeans. Crop Sci 11:242–244

Bonato ER, Vello NA (1999) *E6*, a dominant gene conditioning early flowering and maturity in soybeans. Genet Mol Biol 22:229–232

Buzzell RI (1971) Inheritance of a soybean flowering response to fluorescent-daylength conditions. Can J Cytol 13:103–106

Buzzell RI, Voldeng HD (1980) Inheritance of insensitivity to long daylength. Soyb Genet Newsl 7:26–29

Cober ER, Tanner JM, Voldeng HD (1996) Genetic control of photoperiod response in early-maturing, near-isogenic soybean lines. Crop Sci 36:601–605

Cober ER, Voldeng HD (2001) A new soybean maturity and photoperiod-sensitivity locus linked to *E1* and *T*. Crop Sci 41:698–701

Cregan PB, Jarvic T, Bush AL, Shoemaker RC, Lark KG, Kahler AL, Kaya N, VanToai TT, Lohnes DG, Chung J, Specht JE (1999) An integrated genetic map of the soybean genome. Crop Sci 39:1464–1490

Culley TM, Klooster MR (2007) The cleistogamous breeding system: a review of its frequency, evolution, and ecology in angiosperms. Bot Rev 73:1–30

Darwin C (1897) The different forms of flowers on plants of the same species. D. Appleton and Company, NY, pp 1–352

Fehr WR, Caviness CE, Burmood DT, Pennington JS (1971) Stage of development descriptions for soybeans, *Glycine max* (L.) Merrill. Crop Sci 11:929–931

Goto K, Yamamoto T (1972) Studies on cool injury in bean plants. Part 3. Abnormalities in the reproductive processes related to pod dropping as affected by cool temperature before anthesis in soybean plants (in Japanese). Res Bull Hokkaido Natl Agric Exp Stn 100:14–19

Hayashi T, Awata T (2006) Interval mapping for loci affecting unordered categorical traits. Heredity 96:185–194

Holmberg SA (1973) Soybeans for cool temperate climates. Agric Hort Genet 31:1–20

Hume DJ, Jackson AKH (1981) Pod formation in soybeans at low temperatures. Crop Sci 21:933–937

Japan Beans and Peas Foundation (1991) Legume cultivars in Hokkaido (in Japanese), 2nd edn, Hokkaido Kyodokumiai Tsushinsha, Sapporo, Japan, pp 147 and 155

Kamiya M, Kiguchi T (2003) Rapid DNA extraction method from soybean seeds. Breed Sci 53:277–279

Kawaguchi M, Motomura T, Imaizumi-Anraku H, Akao S, Kawasaki S (2001) Providing the basis for genomics in *Lotus japonicus*: the accessions Miyakojima and Gifu are appropriate crossing partners for genetic analyses. Mol Genet Genomics 266:157–166

Lander ES, Green P, Abrahamson J, Barlow A, Daly MJ, Lincoln SE, Newburg L (1987) MAPMAKER: an interactive computer package for constructing primary genetic linkage map of experimental and natural populations. Genomics 1:174–181

Mano Y, Kawasaki S, Takaiwa F, Komatsuda T (2001) Construction of a genetic map of barley (*Hordeum vulgare* L.) cross 'Azumamugi' x 'Kanto Nakate Gold' using a simple and efficient amplified fragment-length polymorphism system. Genome 44:284–292

Matsumura H, Liu B, Abe J, Takahashi R (2008) AFLP mapping of soybean maturity gene *E4*. J Hered 99:193–197

McBlain BA, Bernard RL (1987) A new gene affecting the time of flowering and maturity in soybean. J Hered 78:160–162

Molnar SJ, Rai S, Charette M, Cober ER. (2003) Simple sequence repeat (SSR) markers linked to *E1*, *E3*, *E4*, and *E7* maturity genes in soybean. Genome 46:1024–1036

Murray MG, Thompson WF (1980) Rapid isolation of high molecular weight plant DNA. Nucl Acid Res 8:4321–4325

Ray JD, Hinson K, Manjono JEB, Maro MF (1995) Genetic control of long-juvenile trait in soybean. Crop Sci 35:1001–1006

Saindon G, Voldeng HD, Beversdorf WD, Buzzell RI (1989) Genetic control of long daylength response in soybean. Crop Sci 29:1436–1439

Shemske DW (1978) Evolution of reproductive characteristics in *Impatiens* (Balsaminaceae): the significance of cleistogamy and chasmogamy. Ecology 59:596–613

Song Q J, Marek LF, Shoemaker RC, Lark KG, Concibido VC, Delannay X, Specht JE, Cregan PB (2004) A new integrated genetic linkage map of the soybean. Theor Appl Genet 109:122–128

Sunada K, Ito T (1982) Soybean grain quality as affected by low temperature treatments in plants (color of hilum, seed coat cracking) (in Japanese). Report of the Hokkaido Branch, Crop Science Society of Japan and Hokkaido Branch, the Japanese Society of Breeding, vol 22, p 34

Takahashi R, Abe J (1999) Soybean maturity genes associated with seed coat pigmentation and cracking in response to low temperatures. Crop Sci 39:1657–1662

Takahashi R, Asanuma S (1996) Association of *T* gene with chilling tolerance in soybean. Crop Sci 36:559–562

Takahashi R, Benitez ER, Funatsuki H, Ohnishi S (2005) Soybean maturity and pubescence color genes improve chilling tolerance. Crop Sci 45:1387–1393

Takahashi R, Kurosaki H, Yumoto S, Han OK, Abe J (2001) Genetic and linkage analysis of cleistogamy in soybean. J Hered 92:89–92

Van Ooijen JW, Boer MP, Jansen RC, Maliepaard C (2002) MapQTL® 4.0, Software for the calculation of QTL positions on genetic maps. Plant Research International, Wageningen, the Netherlands

Watanabe S, Tajuddin T, Yamanaka N, Hayashi M, Harada K (2004) Analysis of QTLs for reproductive development and seed quality traits in soybean using recombinant inbred lines. Breed Sci 54:399–407

Zeng ZB (1993) Theoretical basis for separation of multiple linked gene effects in mapping quantitative trait loci. Proc Natl Acad Sci USA 90:10972–10976