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Identification and mapping of cleistogamy genes in barley

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Abstract Cleistogamy is a closed type of flowering with ensured self-pollination and an important trait to study evolutionary development in flower organs, reproduction systems, gene flow, and disease control. Still, very limited information is available about the genetic control and regulatory mechanism of this trait in barley. In this work, from the eight crosses between cleistogamous and chasmogamous accessions, five crosses generated chasmogamous F_1 plants and their F_2 plants segregated as 3 chasmogamous:1 cleistogamous, whereas three crosses generated cleistogamous F_1 plants, and their F_2 plants segregated as 1 chasmogamous:3 cleistogamous. Although a single gene was responsible for the control of cleistogamy in these two groups of crosses, the direction of dominance was opposite, suggesting two genes, *cly1* and *Cly2*, for the genetic control of cleistogamy in barley. Epistatic type of gene interaction between the two loci was detected. In the analysis of 99 recombinant inbred lines of 'Azumamugi' × 'Kanto Nakate Gold' and doubled haploid lines of 'Harrington' × 'Mikamo Golden', where in both crosses F_1 was chasmogamous, the *cly1* locus has been mapped on chromosome 2HL. Using the analysis of the F_2 population of 'Misato Golden' and 'Satsuki Nijo' where F_1 was cleistogamous, the *Cly2* locus was mapped in the same region of chromosome 2HL. Because the *cly1* and *Cly2* loci were mapped in the same region in these three different mapping populations, it was concluded that the expression of cleistogamy is under the control of two tightly linked genes or different alleles of the same gene.

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Introduction

Cleistogamy is self-fertilization of unopened flowers and is the opposite of chasmogamy, in which pollination (self- or cross-) occurs in open flowers. Maheshwari (1962, cited in Lord 1981) supported Darwin's theory (1877, cited in Lord 1981) that cleistogamous (CL) flowers were modified forms of chasmogamous (CH) flowers adapted for self-pollination and represent an inherited, alternative mode of reproduction. Lord (1981) suggested four groups of CL plants: (1) preanthesis cleistogamy, where bud pollination occurs followed by anthesis; (2) pseudocleistogamy, where no morphological differences occur between CL and CH flowers other than a lack of expansion and anthesis in CL flowers and could be resulted by environmental factors; (3) complete cleistogamy, where some species produce only CL flowers; and (4) "true" cleistogamy, where various floral dimorphisms result from divergent developmental pathways in a single species or individual.

The major morphological differences between two flowering types in barley were described by Briggs (1978) and Reid (1985), who summarized available data and concluded that the large size of lodicules—in the case of the chasmogamy phenotype—pushed the palea and lemma apart, helping all three anthers to emerge from the florets, while CL plants have small lodicules. With florets

Table 1 List of STS and SSR markers mapped in recombinant inbred lines (RILs) of 'Azumamugi' (*AZ*) × 'Kanto Nakate Gold' (*KNG*) and/or in the F_2 population of 'Misato Golden' (*MG*) × 'Satsuki Nijo' (*SN*)

STS marker	Primer sequences (5′–3′)	Annealing temperature (°C)	$MgCl_2$ (mM)	Extension time (s)	Size of PCR product (kb)				Restriction enzyme	Restriction pattern (allele)			
					MG	SN	AZ	KNG		MG	SN	AZ	KNG
ABC165	CAGTGCATTGTCCCCGGGTG TCATGCTCTCGAGCGTGAAGC	58.0	2	60	0.9	0.9	0.9	0.9	*Ava*I	M	S	M	M
MSU21	GGTCTTTCATGTACCTACC CGAGCTCCTGTCGAGG	55.0	1.5	60	0.5	0.7	0.7	0.5	–	–	–	–	–
e11m19-3STS	TTTTCACTTCAGTACTTCGCATCG CAGCCATCCACAAAGTATGCTG	55.0	2	60	0.7	0.7	0.7	0.7	*Mse*I	M	S	S	M
ABC153	TTTACAATGGGGGGTTCTTCC GGCCATGAGCGTGGGCGCTG	58.0	2	60	0.4	0.4	0.4	0.4	*Scr*F1	M	S	S	M
HvCSG	CACTTGCCTACCTCGATATAGTTTGC GTGGATTCCATGCATGCAATATGTGG	55.0	2	90	0.34	0.34	0.32	0.34	–	–	–	–	–
ABC317	ACCAAACTATTTAACTCTGGGTGG GGAAGCCAAATCTCCGGGTC	56.0	1.5	90	2.0	2.0	1.1	2.0	–	–	–	–	–
MWG866	CATGCCCAATGTTGATCGGAG CCCTTGACCTGATTCGAGATCG	54.4	1.5	30	0.5	0.5	0.5	0.5	*Alu*I	M	M	M	K
MWG2200	AACGAAGCAAGATGGGGAGC TTGGCGTGGATCAGCGCGTC	60.0	1.5	30	0.6	-	0.6	0.6	*Hin*cII	–	–	A	K

remaining closed, self-pollination is inevitable. Honda et al. (2003) also showed association between small lodicules and cleistogamy in barley. Thus, based on these observations, barley belongs to the "true" cleistogamy group in Lord's (1981) classification. Also, several studies reported that wild barley, *Hordeum vulgare* ssp. *spontaneum* (C. Koch) Thell, has open CH flowers, while cultivated barley, *H. vulgare vulgare*, ssp. has both CH and CL plants (Johansen and Bothmer 1994; Kuraichi et al. 1994; Abdel Ghani et al. 2002).

Stebbins (1974, cited in Lord 1981) predicted that cleistogamy could provide a comparative system to study evolution of diverse floral morphologies and breeding systems (in particular taxonomic groups) and provide an example of the importance of this trait for general biology. From a practical point of view, CL phenotypes could be potentially very important for disease resistant projects in barley breeding, providing structural barriers for diseases appearing during the flowering time, such as loose smut and *Fusarium* head blight (Hughes 1996; Yoshida et al. 2001). CL plants could also be important for better control of genetically modified lines of agriculturally important crops (Daniell 2002). For these reasons a better understanding of the genetic control of cleistogamy is necessary.

Genetic control of cleistogamy has been studied in a several species. In barley, Kuraichi et al. (1994) reported that the majority of Japanese two-rowed cultivars has a closed type of flowering. They studied the segregation of $BC_1F_{1,2}$ lines of a cross between 'Misato Golden' and 'Satsuki Nijo' and suggested that a single gene controls cleistogamy. In durum wheat, Chhabra and Sethi (1991) reported that cleistogamy is under control of single gene, and the CH allele is dominant over the CL one. They noted that cleistogamy was rather associated with poorly developed lodicules and stiff perianth, but flower phenotypes were unaffected by environmental conditions. In rice, Nagao and Takahashi (1963) identified a CL mutant with compact panicles, small spikelets, and relatively short height. The authors determined that this mutant has abnormal glumes in which the lower parts cannot be differentiated, and the lemma and palea are united. As a result, the spikelets are tightly held in at flowering time while having normal swelled lodicules. The authors reported that the single recessive gene *d7* determined this mutant phenotype, and that the gene was possibly linked to the gene *d6* on chromosome 4 (Nagao and Takahashi 1963). In soybean, Takahashi et al. (2001) using analysis of F_1 plants, F_2 populations, and their F_3 families suggested that CH is dominant to CL, and CL is under the control of a minimum of two genes with epistatic interactions. Furthermore, the authors noted that CL gene was closely linked to one of maturity genes responsible for insensitivity to incandescent, long day length.

In this report, in addition to Kuraichi et al. (1994), we analyzed different populations of several crosses between CL and CH varieties, including two CH wild lines. We identified the number and location of cleistogamy genes and developed DNA markers closely linked to the genes, which may be valuable in projects involving cleistogamy in barley.

Materials and methods

Plant materials and phenotype evaluation

Two-rowed Japanese varieties 'Kanto Nakate Gold', 'Mikamo Golden', 'Misato Golden' and 'Satsuki Nijo', two-rowed American variety 'Harrington', six-rowed Japanese variety 'Azumamugi', and wild lines PI 284755 [received from Dr. K. Matsui, National Institute of Crop Science, (NICS) Tsukuba, Japan) and OUH602 (received from Okayama University, Kurashiki, Japan] were used as parents. Ninety-nine recombinant inbred lines (RILs) were generated from the cross of 'Azumamugi' and 'Kanto Nakate Gold', whose molecular linkage maps were constructed previously (Mano et al. 2001). One hundred and twenty nine doubled haploid (DH) lines were produced by the *Hordeum bulbosum* method (Kasha and Kao 1970) from the cross of 'Harrington' and 'Mikamo Golden'. The parents, their F_1 and F_2 progenies, and DH lines were planted in rows 15 cm apart and with 40 cm between rows in the fields of NICS, Tsukuba. The RILs were grown 20 cm apart with 80 cm between rows in the field of National Institute of Agrobiological Sciences, Tsukuba. CL and CH plants were assessed visually in the field, and plants with emerged anthers from florets during the pollination time were recorded as CH. Wheat (Chinese Spring)–barley (Betzes) chromosome addition lines (Shepherd and Islam 1981) were kindly provided by Dr. A.K.M.R. Islam, University of Adelaide.

DNA marker analysis and mapping procedure

Plant DNA was extracted as described by Komatsuda et al. (1998). Amplified fragments length polymorphism (AFLP) analysis using *Eco*RI (e01–e11) and *Mse*I (m01–m64) primers were performed as described in Mano et al. (2001). Bulked segregant analysis using the AFLP method was performed according to Michelmore et al. (1991). An AFLP fragment, e11m19-3, was cloned by using a TOPO TA cloning kit (Invitrogen). DNA sequences were determined using ABI 310 sequencer and ABI BigDye Terminator kit, version 2.0. DNA sequences of restricted fragments length polymorphism (RFLP) probes (Table 1) were obtained from the GrainGenes database (Graner et al. 1991; Kleinhofs et al. 1993; Blake et al. 1996). Primers were designed using fast polymerase chain reaction (PCR) (formerly Oligos version 8.1., http://www.biocenter.helsinki.fi/bi/bare-1_htm;; Kalendar 2001). PCR was performed in 96-well microtiter plates using a GeneAmp 9700 Thermal Cycler (Applied Biosystems, Foster City, Calif., USA). Each PCR (10 μl) consisted of 0.2 μM each primer, 0.05 U Ex *Taq* DNA polymerase (Takara), 1× *Taq* buffer, 0.2 mM each dNTP, 1.5–2.0 mM $MgCl_2$ (Table 1), and 20 ng DNA. The PCR amplification consisted of an initial denaturation step of 5 min at 94°C, followed by 30 cycles of three steps: denaturation for 30 s at 94°C, annealing for 60 s at 54.4–60.0°C, and extension for 30–60 s at 72°C (Table 1). A final extension step for 5 min at 72°C was performed. Primer sequences and PCR conditions for MSU21 were obtained from Shin et al. (1990) and for SSR marker HvCSG, from Becker and Heun (1995). Linkage analyses were performed using MAPMAKER, version 3.0 (Lander et al. 1987). Kosambi's function was used to obtain the genetic distances in centiMorgans (Kosambi 1944).

Results

Phenotype of parents, F_1, and segregating populations

Field tests revealed that 'Kanto Nakate Gold', 'Mikamo Golden' and 'Misato Golden' were CL type, and 'Azumamugi', 'Harrington', 'Satsuki Nijo', OUH602 and PI 284755 were CH-type flowering (Table 2). In three consecutive years, these accessions were tested in various places in the fields of NICS, Tsukuba, and the same phenotype was recorded for all accessions during all these years.

In the F_1 generation, plants from CL × CL crosses showed CL phenotype and plants from CH × CH crosses showed CH phenotype (Table 3). Five CL × CH crosses produced CH-type F_1 plants, and three CL × CH crosses involving 'Satsuki Nijo' produced CL-type F_1 plants. The phenotype of F_1 plants of reciprocal crosses (except not tested OUH602 × 'Kanto Nakate Gold' and 'Harrington' × 'Mikamo Golden') was the same, indicating that cytoplasmatic genes were not involved in the genetic control of cleistogamy.

The segregation data of F_2 are shown in Table 3. In CL × CL crosses all F_2 plants were CL type, and in CH × CH crosses all F_2 plants were CH type. In CL × CH crosses F_2 plants showed two types of segregation. In three crosses where F_1 showed CL phenotypes, F_2 segregated 3 CL:1 CH. The segregation pattern indicated that CL is under the control of single dominant gene. In contrast, in the five crosses having the CH phenotype in the F_1, the F_2 segregated 1 CL:3 CH, indicating that cleistogamy is under the control of single recessive gene (Table 3). In the 'Azumamugi' × 'Kanto Nakate Gold' cross, the segregation of F_2 plants was significantly different from the expected 1:3 ratio and did not fit a 7:9 ratio either (χ^2=10.6, 0.001<*P*<0.005). Segregation distortion has previously been noted in the region of the chromosome (Mano et al. 2001).

Table 2 Grouping of barley accessions by phenotype and suggested genotype

Accessions	Phenotype[a]	Genotype
'Kanto Nakate Gold'	CL	*cly1Cly2*
'Mikamo Golden'	CL	*cly1Cly2*
'Misato Golden'	CL	*cly1Cly2*
'Azumamugi'	CH	*Cly1Cly2* or *Cly1cly2*
'Harrington'	CH	*Cly1Cly2* or *Cly1cly2*
OUH602[b]	CH	*Cly1Cly2* or *Cly1cly2*
PI284755[b]	CH	*Cly1Cly2* or *Cly1cly2*
'Satsuki Nijo'	CH	*cly1cly2*

[a]*CL* Cleistogamous, *CH* chasmogamous
[b]Wild barley, *Hordeum vulgare spontaneum* ssp.

Genetic mapping

Using 99 RILs of 'Azumamugi' × 'Kanto Nakate Gold', *cleistogamy 1* was mapped to the telomeric region of chromosome 2HL (Fig. 1a). Since the cleistogamy gene was recessive, the locus symbol was designated as *cly1* (Fig.1a), where the allele of 'Azumamugi' was *Cly1* and allele of 'Kanto Nakate Gold' was *cly1*. For better saturation of the gene region, five STS markers along with the SSR marker HvCSG (Table 1) were added in the chromosome 2HL of 'Azumamugi' × Kanto Nakata Gold genetic map (Fig. 1a). The *cly1* gene cosegregated with MSU21 and e11m19-3. The AFLP marker e11m19-3 was cloned, sequenced, and converted to a STS marker e11m19-3STS. The location of e11m19-3STS on chromosome 2H was confirmed by analyzing wheat–barley addition lines (Fig. 2). The PCR product of e11m19-3STS was present in barley variety Betzes and Chinese Spring-Betzes chromosome 2H addition line and absent in wheat variety Chinese Spring and in all the other addition lines. The allelism of e11m19-3 and e11m19-3STS was confirmed by testing 'Azumamugi' × 'Kanto Nakate Gold' RILs. The AFLP marker e07m34-2 was identified by bulked segregant analysis using 704 *Eco*RI/*Mse*I primer combinations, and mapped 1.6 cM from the *cly1* locus. Thus, the e07m34-2 and the HvCSG/ABC153 cluster flanked the *cly1* locus. The *cly1* locus was mapped at the same position in 'Harrington' × 'Mikamo Golden' DH population (Fig. 1a). In this population, unlike to 'Azumamugi' × 'Kanto Nakate Gold', two recombinant plants were identified between MSU21 and the *cly1* locus, leading to the distance of 0.9 cM.

The *cleistogamy 2* gene was mapped using 'Misato Golden' × 'Satsuki Nijo' $F_{2:3}$ lines (Fig. 1c). F_2 plants of 'Misato Golden' × 'Satsuki Nijo', followed by F_3 analysis, segregated in 41 homozygous CL, 73 heterozygous, and 36 homozygous CH, fitting 1:2:1 segregation ratio (χ^2=0.44, 0.75<*P*<0.9). The e11m19-3STS, ABC165, MSU21, ABC153, and MWG2200 markers were used for the construction of the linkage map. The order of

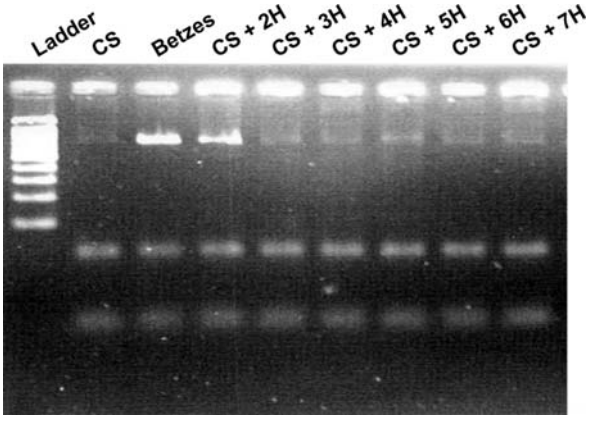

Fig. 2 Chromosomal location of e11m19-3STS marker using Chinese Spring (*CS*, wheat)-*Betzes* (barley) chromosome addition lines. *Ladder* is DNA ladder (100 bp). Lines from *CS + 2H* to *CS + 7H* are wheat–barley chromosome addition lines

Table 3 Segregation in F_2 and fixed generations for CL and CH plants in barley

Crosses	F_1 phenotype	Population	CL	CH	Total	*P*-value range		
						χ^2 (3:1)	χ^2 (1:3)	χ^2 (1:1)
CL × CL crosses								
'Misato Golden' × 'Kanto Nakate Gold'	CL[a]	F_2	196	0	196	–	–	–
CH × CH crosses								
'Satsuki Nijo' × 'Azumamugi'	CH[a]	F_2	0	191	191	–	–	–
'Satsuki Nijo' × PI 284755	CH[a]	F_2	0	193	193	–	–	–
PI 284755 × 'Azumamugi'	CH[a]	F_2	0	195	195	–	–	–
CL × CH crosses								
'Kanto Nakate Gold' × 'Satsuki Nijo'	CL[a]	F_2	119	45	164	0.30–0.50	–	–
'Misato Golden' × 'Satsuki Nijo'	CL[a]	F_2	114	36	150	0.75–0.90	–	–
'Satsuki Nijo' × 'Misato Golden'	CL[a]	F_2	139	52	191	0.30–0.50	–	–
'Kanto Nakate Gold' × PI 284755	CH[a]	F_2	51	139	190	–	0.50–0.70	–
'Misato Golden' × 'Azumamugi'	CH[a]	F_2	48	136	184	–	0.70–0.80	–
'Misato Golden' × PI 284755	CH[a]	F_2	35	115	150	–	0.50–0.80	–
OUH602 × 'Kanto Nakate Gold'	CH[b]	F_2	155	448	603	–	0.50–0.80	–
'Azumamugi' × 'Kanto Nakate Gold'	CH[a]	F_2	62	131	193	–	0.02–0.05	–
'Azumamugi' × 'Kanto Nakate Gold'	CH[a]	RIL	37	62	99	–	–	0.01–0.02
'Harrington' × 'Mikamo Golden'	CH[b]	DH[c]	63	66	129	–	–	0.70–0.80

[a]F_1 plants showed the same phenotype in reciprocal crosses
[b]F_1 plants were not tested reciprocally
[c]*DH* Doubled haploid

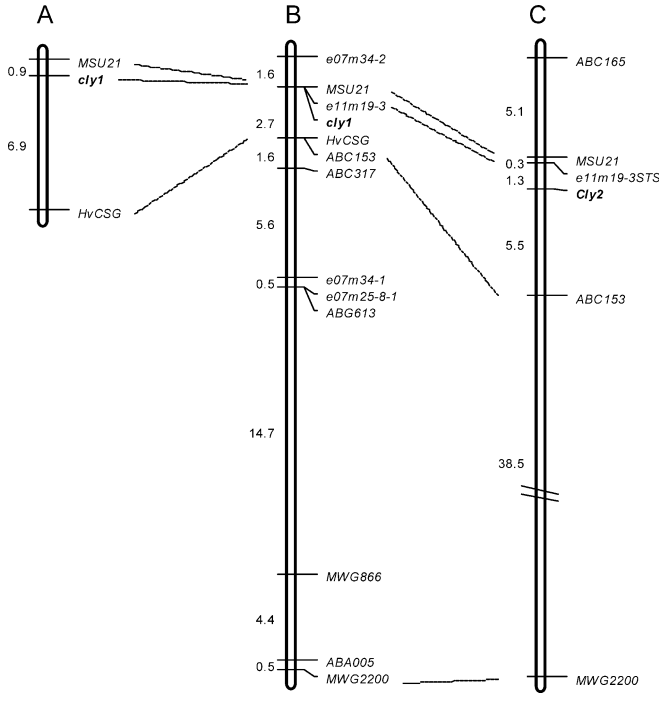

Fig. 1a–c Linkage maps of cleistogamy genes in chromosome 2H of barley. Map distances are shown in centiMorgans **a** 'Harrington' × 'Mikamo Golden' doubled haploid population map, including the *cly1* locus. **b** 'Azumamugi' × 'Kanto Nakate Gold' RILs map, including the recessive cleistogamy gene *cly1*. **c** 'Misato Golden' × 'Satsuki Nijo' F_2 population map, including the dominant cleistogamy gene *Cly2*

cleistogamy 2 and DNA markers was congruent with the maps of 'Azumamugi' × 'Kanto Nakate Gold' RILs and 'Harrington' × 'Mikamo Golden' DH lines (Fig. 1). Although the location of the gene was identical with *cly1*, a dominant gene controlled the cleistogamy in this population, and therefore, the locus symbol was designated as *Cly2*. The allele of 'Misato Golden' was designated as *Cly2*, and the allele of 'Satsuki Nijo' as *cly2*. Suggested genotypes of all the parental lines are summarized in Table 2.

Discussion

Genetic mapping of CL genes and a hypothesis for genetic control of cleistogamy

To our knowledge, this is the first report where cleistogamy genes were positioned in the genetic maps of barley, and possibly, in cereals. The segregation pattern of all CL × CH populations showed that a single gene was responsible for the control of cleistogamy in these crosses. However, the opposite action of dominant genes in these crosses (Table 3) also suggests a possibility that two different genes, *cleistogamy 1* and *cleistogamy 2*, control the cleistogamy in barley. In a latter case, the results indicated that the *Cly2*– *cly2* pair is hypostatic to the *Cly1* gene of CH plants. In Fig. 3, we suggest a hypothetical model for the mechanism of regulation for a type of flowering in barley. According to this model, in the absence of the *Cly1* allele, products of the *Cly2* allele act

as inhibitors for the open type of flowering. *Cly1* suppresses the action of *Cly2*, which may play regulatory role in transition from close to open type of flowering (Fig. 3).

No CL plants were segregated from F_2 of 'Satsuki Nijo' × 'Azumamugi' and 'Satsuki Nijo' × PI284755 (Table 3). Therefore, if 'Azumamugi' and PI284755 have the *Cly1Cly2* genotype, these results can be explained by the hypothesis of tight linkage between the *cly1* and *Cly2* loci. Both *cly1* and *Cly2* have been mapped in the telomeric region of chromosome 2HL in barley. The two cleistogamy genes have been flanked by MSU21 and ABC153, which have been mapped to chromosome 2HL in several mapping projects of barley (Kleinhofs et al. 1993; Thomsen et al. 1997; Costa et al. 2001). The closest DNA marker to *cly1* and *Cly2* was e11m19-3STS; the marker cosegregated with the *cly1* locus in RILs of 'Azumamugi' × 'Kanto Nakate Gold' and was located 1.3 cM apart from the *Cly2* locus in F_2 population of 'Misato Golden' × 'Satsuki Nijo' cross. The results agree with the hypothesis of tight linkage between the *cly1* and *Cly2* loci. The occurrence of gene duplication and location of two genes in tandem is well spread in diploid species (Alberts et al. 1989) and suited for the explanation of our results. In this work we tentatively applied the two-genes model for Figs.1 and 3 and Table 2.

In an alternative way, the results can be explained by the possibility that three or more alleles were differentiated at the single locus. For instance, 'Misato Golden' and 'Kanto Nakate Gold' may have a *Cly.b* allele of the gene that produces multimer proteins (e.g., a dimer), and 'Satsuki Nijo' has a null allele, and the other lines have *Cly.a* alleles that produce proteins able to interact with *Cly.b* proteins to form a nonfunctional multimer. If a certain critical concentration of functional CLY oligomers were necessary for cleistogamy to occur, then the formation of nonfunctional heteromeric complexes in *Cly.a*/*Cly.b* plants would explain why *Cly.b* alleles are recessive to *Cly.a* in some backgrounds.

Thus, in comparison to the results in durum wheat (Chhabra and Sethi 1991) and rice (Nagao and Takahashi 1963) where a single gene controlled the cleistogamy, two tightly linked cleistogamy genes or single gene with three or more alleles are possible in barley. In addition, the loci were genetically mapped, and new molecular markers were developed, opening the prospect of further studies of cleistogamy in relative species. In soybean, Takahashi et al. (2001) identified a minimum of two cleistogamy genes

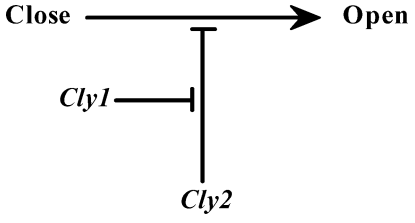

Fig. 3 A hypothetical model of the regulation for a type of flowering in barley

with epistatic interaction and noted that temperature affects the cleistogamy phenotype. In contrast to soybean, the environment did not alter the cleistogamy or chasmogamy in barley, indicating the stable expression of the genes under various field conditions in the three consecutive years.

Genetic factors associated with CL genes

There are several morphological factors found to be associated with CL. Honda et al. (2003), using B_4F_3 plants of 'Misato Golden' × 'Satsuki Nijo' cross, reported that CL is associated with small lodicules and high density of spike. A quantitative trait locus (QTL) for spike density was detected at the interval between e11m19-3 and ABC613, where the *cly1* was detected, in 'Azumamugi' × 'Kanto Nakate Gold' RILs (Sameri and Komatsuda, unpublished data). Also, a mutant gene *zeo1* (zeocriton, dwarf, dense spike) was mapped between MSU21 and ABC153 on chromosome 2H in the Oregon Wolfe barley mapping project (Costa et al. 2001). In our study, the cleistogamy genes were mapped in the same region, suggesting an association between cleistogamy and dense spike. Honda et al. (2003), using F_2 plants of 'Misato Golden' × 'Satsuki Nijo', demonstrated the relationship between CL and low response to auxin treatment and suggested that some of the auxin-related genes may play a key role in the control of cleistogamy.

Different studies of disease resistance also allowed the identification of several QTLs localized in this region (Thomsen et al. 1997; Scheurer et al. 2001; Mesfin et al. 2003). For instance, Mesfin et al. (2003), using crosses between two-rowed 'Fredrickson' and six-rowed 'Stander', identified major QTLs resistant to *Fusarium* head blight (greenhouse conditions), deoxinivalenol accumulation, and heading date (field conditions) with locations just proximal to the ABC153 marker on chromosome 2H. This result agrees with report of Yoshida et al. (2001), which clearly shows that CL correlates with moderate resistance to *Fusarium* head blight in barley, and hence, underscores the importance of cleistogamy in the genetic and breeding programs of barley.

Future implications of the results in practice and theory

Kunzel et al. (2000) compared the distances between genetic and physical maps of barley and divided the barley genome into three types of regions: suppressed (>4.4 Mb per cM), increased (1.0–4.4 Mb per cM), and strongly increased number of recombinations (≤1.0 Mb per cM). According to this grouping, the segment of chromosome 2HL—where the cleistogamy genes were mapped—approximately belongs to the region with increased number of recombinations. The distance of 1.0–4.4 Mb per cM is still rather long, and higher resolution of maps is required. However, recent successes in the construction of

libraries with large sizes of DNA inserts, such as Morex (Yu et al. 2000) and Haruna Nijo (Saisho et al. 2002) BAC DNA libraries, and progress in rice genome sequencing (Saji et al. 2001; Feng et al. 2002; Sasaki et al. 2002) have been promising the feasibility of efficient map-based cloning. The utilization of these approaches may lead to a better understanding of the regulatory mechanism of cleistogamy in barley. It is also important to note that cleistogamy genes were flanked by closely located PCR-based codominant markers. These markers could be effectively used in breeding and genetics projects related to cleistogamy. Particularly, they may facilitate the monitoring of the self-pollination of plants, to ensure seed production in various hybrid generations, to study the resistance to diseases associated with infections of flower organs and may further enlighten the micro- and macro-evolutionary processes of *Hordeum* genera and relative taxonomic groups, including the morphological developments of flower and spike structures.

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