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Genetic mapping of a quantitative trait locus (QTL) that enhances the shoot differentiation rate in *Hordeum vulgare* L.

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Abstract. A quantitative trait locus (QTL) controlling shoot differentiation from immature embryo callus was identified by linkage analysis with morphological and isozyme markers in barley, *Hordeum vulgare* L. Immature embryos were isolated from cvs 'Azumamugi' (difficult to differentiate), 'Kanto Nakate Gold' (easy to differentiate), their hybrids (F_1) and a backcross population derived from a cross 'Azumamugi' × F_1. The embryos were cultured *in vitro* for callus initiation and subsequent shoot differentiation. The shoot differentiation rate was closely associated with ear type (*v* locus), isocitrate dehydrogenase isozyme (*Idh-2*), and esterase isozyme (*Est-11*). These markers were found to reside in a chromosome segment of approximately 30 cM on chromosome 2. Recombination frequency was 9.9% between *v* and a proposed QTL named "*Shd1*" (shoot differentiation), 11.5% between *Idh-2* and *Shd1*, and 21.3% between *Est-11* and *Shd1*. All data showed the *Idh-2*, *v*, *Shd1* and *Est-11* loci to be arranged in this order from proximal to distal on the long arm of chromosome 2.

Key words: *Hordeum vulgare* – Genetic mapping – Isozymes – Quantitative trait loci – Shoot differentiation ability

Introduction

Plant regeneration is under genetic as well as physiological control, and thus the transfer of regeneration genes to recalcitrant cultivars or species is one way to improve the regeneration capacity of plants (as proposed by Kuhlmann and Foroughi-Wehr 1989). The transfer of genes by recurrent backcrosses is practical only when the number of genes which complement the allele of a non-regenerating genotype is one or a few. The mapping of major genes controlling regeneration ability is thus required to achieve a new scheme for improving regeneration ability. Genetic marker-associated regeneration factors should lessen the difficulty of selection in recurrent backcross breeding.

Genotypic differences are manifested by the ability to regenerate from immature barley embryo calli (Hanzel et al. 1985; Lührs and Lörz 1987; Ohkoshi et al. 1991). Diallel analysis has indicated the ability of shoot differentiation to be highly heritable with a high degree of dominance (Komatsuda et al. 1989). Japanese two-rowed barley cultivars produce embryogenic calli from mature embryos and differentiate shoots at higher frequencies than Japanese and foreign six-rowed cultivars (Taniguchi et al. 1991). Ear type (number of kernel rows) is predominantly controlled by a single locus, *V-v*, on chromosome 2; and the genotype *VV* or *Vv* has a two-rowed ear, whereas *vv* has a six-rowed ear (Harlan and Hayes 1920; Griffee 1925). In a diallel analysis Komatsuda et al. (1989) demonstrated that the parent of 'Kanto Nakate Gold', with a high differentiation ability, is two-rowed whereas 'Azumamugi', with a low differentiation ability, is six-rowed. 'Kanto Nakate Gold' has dominant gene(s) for shoot differentiation, while 'Azumamugi' has recessive gene(s). A linkage relationship between the *v* locus and shoot differentiation rate was detected using an F_2 population produced from the cross 'Azumamugi' × 'Kanto Nakate Gold' (Kamatsuda et al. 1991).

In the study presented here, genetical factors controlling the shoot differentiation rate were considered

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to be quantitative trait loci (QTL), and QTL controlling shoot differentiation were mapped using the *V/v* gene and two isozyme markers on chromosome 2. The strategy for transferring QTL to recalcitrant barley cultivars is discussed.

Materials and methods

Plant material

F_1 plants were obtained from a cross betweeen a Japanese six-rowed cv 'Azumamugi' and a Japanese two-rowed cv 'Kanto Nakate Gold' (Komatsuda et al. 1989). A single F_1 plant as a pollen donor was back crossed to 'Azumamugi' to obtain a BC_1F_1 population. In total, 119 BC_1F_1 plants and two cultivars and F_1 plants were grown in the field.

Detection of polymorphism in the parents

Morphological markers

BC_1F_1 plants were scored for ear type, awn length and auricle colour. The recurrent parent 'Azumamugi' possesses recessive alleles for each character, exhibiting a six-rowed ear (*v*), "uzu" or semi-brachytic growth with short awn (*uz*) and non-purple auricle (*pau*). BC_1F_2 seedings were examined for coleoptile length following the method of Takahashi and Yamamoto (1951) to confirm the *Uz-uz* genotype of the plants. From each BC_1F_1 plant 10 BC_1F_2 progenies were again evaluated as a check for ear type and awn length.

Spring and winter habits of growth

Spring/winter habits of growth were determined following the method of Takahashi and Yasuda (1956). Thirty BC_1F_2 progeny from each BC_1F_1 plant, together with F_1, F_2 and their parental cultivars, were grown in a greenhouse under continuous illumination at a temperature maintained between 17 °C and 23 °C. The date when the flag-leaf on the main stem appeared from the leaf sheath below was used as the criterion of the growth habit. 'Kanto Nakate Gold', with a spring habit, possesses two genes for the spring type of growth: a recessive *sh* gene on chromosome 4 and a dominant *Sh2* gene on chromosome 7, thus exhibiting the genotype *shshSh2Sh2* (Takahashi et al. 1983). 'Azumamugi', a winter barley, has the genotype *ShShsh2sh2*. BC_1F_1 plants should thus segregate at a 1:1:1:1 ratio for the genotype *ShShsh2sh2*:*Shshsh2sh2*:*ShShSh2sh2*:*ShshSh2sh2*. In BC_1F_2 progenies, *Sh_Sh2_*, shshSh2_ and *shshsh2sh2* plants show spring-type growth, whereas *Sh_sh2sh2* shows winter-type growth. BC_1F_1 plants with the *ShShsh2sh2* genotype should thus segregate in their progenies at a 0:1 ratio for spring:winter type: *Shshsh2sh2* (1:3), *ShShSh2sh2* (3:1) and *ShshSh2sh2* (13:3). *ShShSh2sh2* and *ShshSh2sh2* genotypes, however, cannot be distinguished due to the closed segregation ratio. To clarify alleles at the *sh* locus of the plants, BC_1F_2 plants showing the winter phenotype were subjected to vernalization and then self-pollinated to produce BC_1F_3 seeds. The BC_1F_3 plants were again tested for the spring/winter habit, and alleles at the *sh* locus were determined.

Isozyme markers

For the analysis of α-amylase (AMY), single kernels of 5-day-old seedlings were ground in 300 µl TRIS-HCl (pH 8.5), incubated for 1 h at 40 °C, centrifuged at 12,000 rpm for 3 min and incubated for 15 min at 70 °C. For all of the other isozymes, single plumules and coleoptiles between 6 and 10 days following germination under dim light at 20 °C were crushed in 100 µl 0.1% 2-mercaptoethanol and centrifuged at 5,000 rpm for 5 min. Aliquots of the supernatant were used for isoelectric focusing and starch gel electrophoreses.

Isoelectric focusing methods used for seed AMY have been described by Takano and Takeda (1985), for leaf anodal esterase (EST) by Ban and Kawada (1989) and for leaf cathodal esterase (EST) by Ban and Kawada (1990). The starch gel electrophoresis method of Benito et al. (1988) was used for separating leaf glucose-6-phosphate dehydrogenase (G6PD), isocitrate dehydrogenase (IDH) and phosphogluconic dehydrogenase (PGD). The starch gel method of Glaszmann et al. (1988) was used for separating leaf alcohol dehydrogenase (ADH), alanine aminopeptidase (AMP), phosphoglucose isomerase (PGI) and shikimate dehydrogenase (SDH).

The staining methods used were those of Takano and Takeda (1985) for AMY, Nielsen and Johansen (1986) for EST and G6PD and Glaszmann et al. (1988) for AMP, ADH, G6PD, IDH, PGD and PGI.

Interparental variation was observed for EST (both anodal and cathodal), IDH, AMP and AMY, but not for ADH, G6PD, PGI, PGD or SDH. EST, IDH, AMP and AMY were used as isozyme markers for genetic analysis. The genotypes of the BC_1F_1 plants used for their isozyme markers were determined by segregation in at least 4 BC_1F_2 seedlings per progeny. BC_1F_1 plants were assumed to be homozygous for the 'Azumamugi' allele at the isozyme locus only if all 4 seedlings were homozygous for the 'Azumamugi' allele: When at least 1 heterozygote or 'Kanto Nakate Gold' homozygote was detected, the BC_1F_1 plant was considered to be heterozygous. Heterozygotes may be misclassified as homozygotes at a probability of 1/256.

Callus culture and shoot differentiation

From each plant 30 immature embryos were cultured for callus initiation and shoot differentiation as described previously (Komatsuda et al. 1989). The shoot differentiation rate, like the germination rate of seeds, is a threshold character, and thus we regarded the ratio of differentiating callus per immature embryo callus as representative of the differentiation ability of the mother plant. The differentiation ability of each F_1 and BC_1F_1 plant was therefore evaluated using the progeny of the following F_2 and BC_1F_2 generations, respectively. Implicit in such a progeny test is a slight underestimation of differentiation ability due to the reduction of the dominance effect by a half, but this does not affect the results of linkage analysis for QTL mapping. All differentiation rate data (p) were transformed to arcsin $\sqrt{p}$ values to improve the normality of distribution prior to the estimation of recombination frequency.

Estimation of recombination frequency

The recombination frequency between a marker locus and QTL in a backcross population was estimated with the methods of Jensen (1989) and Luo and Kearsey (1991) with modification. The method of Jensen (1989) was applied for doubled haploid lines and that of Luo and Kearsey (1991) for doubled haploid and backcrossed populations. However, bias due to the distorted segregation of markers was not considered. In our study, we observed distorted segregation for marker and QTL alleles, and thus a new micro-computer programme suitable for calculating recombination frequency between a skewed marker and skewed QTL alleles, as well as between two skewed markers (Annaka et al. in preparation), written in "C" language was used.

Transfer of differentiation ability by recurrent backcross

BC_1F_1 plants were cross-pollinated with 'Azumamugi'. The ears of BC_2F_1 plants were either self-pollinated to initiate immature

Table 1. Morphological, anthocyanin and isozyme markers used in linkage analysis, their phenotypes and chromosomal locations

Locus	Phenotype	Chromosomal location (reference)
V/v	Two/six rowed	2 (Griffee 1925)
Idh-2	Isocitrate dehydrogenase	2 (Brown and Munday 1982)
Est-11	Esterase	2 (T. Ban, personal communication)
Pau/pau	Purple/colorless auricle	2 (Doney and Woodward 1963)
Uz/uz	Normal/semi-brachytic growth	3 (Takahashi and Yamamoto 1951)
Est-2	Esterase	3 (Nielsen and Frydenberg 1971b)
Sh/sh	Winter/spring habit of growth	4 (Takahashi et al. 1958, Takahashi and Hayashi 1966)
Amp-1	Aminopeptidase	6 (Hart et al. 1980)
Amy1	α-Amylase	6 (Nielsen and Frydenberg 1971a)
Sh2/sh2	Spring/winter habit of growth	7 (Takahashi and Yasuda 1956)

embryo callus cultures for screening shoot differentiation ability or cross-pollinated with 'Azumamugi' to produce BC_3F_1 plants. The BC_3F_1 plants were again self-pollinated to obtain embryos or cross-pollinated with 'Azumamugi' for progressing recurrent backcross and selection.

Results

Segregation of markers

The BC_1F_1 progeny segregated for three morphological marker loci, two physiological marker loci and five isozyme loci (Table 1).

Some plants showed partial seed fertility in lateral florets. However, these lateral florets were completely awn-less, and the heterozygotes (*Vv*) could thus be easily distinguished from homozygous six-rowed plants (*vv*). A progeny test confirmed the classification of the genotypes to be accurate. As shown in Table 2, the frequency of six-rowed plants was twice that of two-rowed phenotypes, indicating the deviation from the expected segregation of 1:1 to be significant.

Uz-uz alleles of BC_1F_1 plants were determined by a typical bimodal curve of awn length; one mode was found at 65–100 mm, and the other at 110–190 mm (curve not shown). The longer group was regarded as being *Uzuz* and the shorter group as *uzuz*. Identical results were obtained from progeny analysis of coleoptile length and awn length.

Anthocyanin pigmentation at the flag-leaf auricle served as a criterion for determining *Pau-pau* alleles in BC_1F_1 plants. The heterozygote for the allele as well as the F_1 plants showed an intermediate purple colour at the auricle. However, colour intensity largely varied among BC_1F_1 plants, so that some plants could hardly be distinguishable from those that were colourless (recessive homozygote). This trait was thus not very suitable as a genetic marker.

As for the spring habit of growth, plants which unfolded the flag-leaf on the main stem within 10 weeks after sowing were regarded as having the spring-type growth habit, whereas later ones, the winter type. Due to the BC_1F_1 base, alleles at the *sh2* locus segregated at just a 1:1 ratio for the homozygote recessive:heterozygote genotypes, whereas alleles at the *sh* locus, at a 2:1 ratio in favour of 'Azumamugi' alleles.

All isozyme alleles showing polymorphism were codominant. Isozymes encoded by *Idh-2* appeared to be dimers (Benito et al. 1988), while those encoded by *Amp-1*, monomers. Many bands of isozymes were encoded by alleles at the *Amy 1* locus, but only one of the multiple bands was polymorphic between two barley cultivars, as has been described by Takano et al. (1988).

Table 2. Segregation of marker genes in a barley BC_1F_1 population from 'Azumamugi' × F_1 ('Azumamugi' × 'Kanto Nakate Gold')

Chromosomal location	Genotype of[a]		Segregation of BC_1F_1 plants[b]		
	Azumamugi	Kanto Nakate Gold	A/A	A/K	χ^2 for 1:1
2	*v*	*V*	79	40	12.78***
2	$Idh\text{-}2^{AZ}$	$Idh\text{-}2^{Ka}$	72	36	12.00***
2	$Est\text{-}11^{Yo}$	$Est\text{-}11^{Cl}$	78	39	13.00***
2	*pau*	*Pau*	54	64	0.85
3	*uz*	*Uz*	53	65	1.22
3	$Est\text{-}2^{Fr}$	$Est\text{-}2^{Dr}$	58	60	0.03
4	*Sh*	*sh*	63	33	9.37**
6	$Amp\text{-}1^{Az}$	$Amp\text{-}1^{Ka}$	65	43	4.48*
6	$Amy\text{-}1^{3}$	$Amy\ 1^{1}$	76	38	12.67***
7	*sh2*	*Sh2*	48	49	0.01

*, **, *** Significant at the 5%, 1% and 0.1% probability levels, respectively

[a] Az, Ka: tentatively named

[b] A/A, homozygous for 'Azumamugi' allele; A/K, heterozygous as F_1 genotype

Table 3. Linkage between isozyme and morphological loci in BC_1F_1 progenies of the cross 'Azumamugi' × F_1 ('Azumamugi' × 'Kanto Nakate Gold')

Loci	Segregation of BC_1F_1 progenies[a]				χ^2 for linkage	Recombination (%)
	(AA, AA)	(AA, AK)	(AK, AA)	(AK, AK)		
Idh-2 ~ *v*	70	2	3	33	88.93***	4.6 ± 2.0
v ~ *Est-11*	63	14	15	25	36.08***	24.8 ± 4.0
Idh-2 ~ *Est-11*	56	16	17	19	16.33***	30.6 ± 4.4

*** Significant at the 0.1% probability level
[a] A/A, homozygous for 'Azumamugi' allele; A/K, heterozygous as F_1 genotype

Est-11 alleles coded cathodal esterase enzymes showing rather weak bands. The *Est-11*^{Cl} allele encoded a family of three narrow bands, whereas the *Est-11*^{Yo} allele encoded a thick band possessing an iso-electric point that differed considerably from the three narrow ones (see Ban and Kawada 1990).

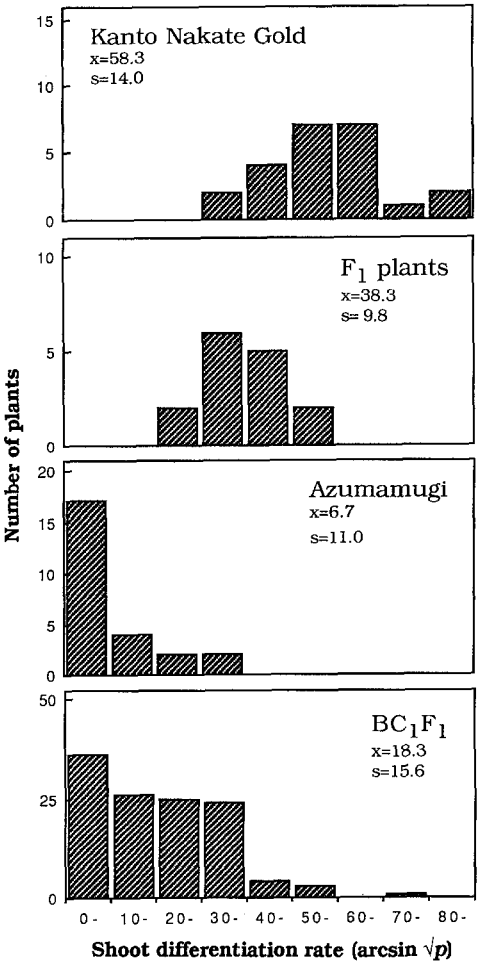

Fig. 1. Shoot differentiation rates of immature embryo callus in barley cv 'Kanto Nakate Gold', cv 'Azumamugi', F_1 and BC_1F_1 plants. The BC_1F_1 population was obtained from a cross of 'Azumamugi' with F_1

Of the ten markers tested, six showed distorted segregation (Table 2). Each of *v*, *Idh-2* and *Est-11* on chromosome 2 and *Amy 1* on chromosome 6 roughly segregated at a 2:1 ratio in favour of the 'Azumamugi' alleles.

Linkage between marker loci

Linkage relationships between the *Idh-2*, *v*, and *Est-11* loci were found (Table 3). Benito et al. (1988) found the recombination frequency between the *Idh-2* and *v* locus to be 13.18% in an F_2 population; it was much less in our study – 4.6%. The *v* locus is linked with the *Est-11* locus at a recombination percentage of 24.8, which is consistent with the 29.0% estimated by T. Ban (personal communication). The linkage map of Benito et al. (1988) showed *Idh-2* to be proximal on the long arm of chromosome 2, with the *v* locus being distal. Based on this and the present results, the following gene order was proposed: *Idh-2* – *v* – *Est-11*, from the proximal to distal end of the long arm of chromosome 2 (Fig. 3). *Amp-1* alleles were found to be inherited independent of *Amy 1* alleles (data not shown).

Shoot differentiation

The mean differentiation ability of the F_1 plants exceeded the average of the parental cultivars, which agrees with previous results that indicated a positive dominance effect on shoot differentiation (Komatsuda et al. 1989). The mean differentiation ability of BC_1F_1 plants was less than the mean value of 'Azumamugi' and F_1 plants (Fig. 1), possibly due to the distorted segregation of QTL related to shoot differentiation resulting in the prevalence of the 'Azumamugi' allele in the BC_1F_1 population.

Association between genetic markers and shoot differentiation

Shoot differentiation ability was significantly associated with ear type (Table 4). The histograms in Fig. 2 show the association between the *v* locus and differentiation ability. The mean differentiation ability of BC_1F_1

Table 4. Comparison of shoot differentiation rates (arcsin $\sqrt{p}$) between BC_1F_1 plants containing different genetic markers

Chromosome number	Marker locus	Mean ± standard error[a]		Variation[b] (%)
		A/A	A/K	
2	*v*	12.6 ± 1.4	29.5 ± 2.3***	65.4
	Idh-2	12.9 ± 1.5	29.2 ± 2.6***	61.4
	Est-11	15.1 ± 1.7	25.1 ± 2.3***	46.2
	pau	18.9 ± 1.7	17.3 ± 2.2	
3	*Est-2*	18.9 ± 2.1	18.3 ± 2.0	
	uz	13.5 ± 1.7	21.6 ± 2.0**	20.7
4	*sh*	18.3 + 1.9	16.7 + 2.5	
6	*Amp-1*	19.1 ± 1.9	17.1 ± 2.5	
	Amy 1	18.2 ± 1.8	19.1 ± 2.5	
7	*sh2*	18.5 ± 2.3	17.2 ± 2.0	

, * means significantly differ at the 1% and 0.1% probability levels in the *t*-test, respectively

[a] A/A, homozygous for 'Azumamugi' allele; A/K, heterozygous as F_1 genotype

[b] Percentage of the portion of genetic variance associated with allelic differences at marker loci in a BC_1F_1 population

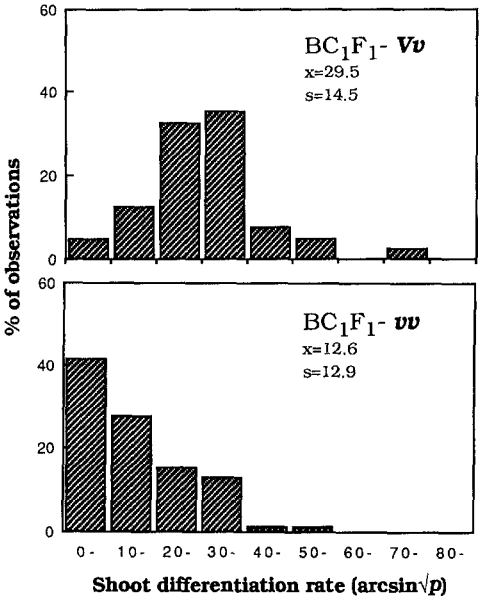

Fig. 2. Distribution of the shoot differentiation rate based on F_1 (*Vv*) and 'Azumamugi' (*vv*) genotypes for ear type in BC_1F_1 plants

plants carrying *Vv* and *vv* was 29.5 ± 2.3 and 12.6 ± 1.4, respectively. These two values were found to be highly significant in the *t*-test. The association of the differentiation rate with the *V-v* alleles explains 65.4% of the total genetic variance in the BC_1F_1 population. The *Idh-2* and *Est-11* loci, linked with the *v* locus, showed an association with the shoot differentiation rate (Table 4). The association of the differentiation rate with these two isozyme alleles explains 61.4% and 46.2%, respectively, of the total genetic variance in the BC_1F_1 population, thereby indicating that the *v* locus is associated primarily with shoot differentiation ability. The high degree of association indicates that the alleles at the *v* locus have a pleiotrophic effect on shoot differentiation or that the *v* locus is linked with a QTL "*Shd1*" (shoot differentiation). The alleles of *Shd1*, which originated from 'Azumamugi' and 'Kanto Nakate Gold', were designated *Shd1A* and *Shd1K*, respectively.

QTL mapping of differentiation ability

The proportion of heterozygotes for the *Shd1* locus in BC_1F_1 plants was estimated to be 31.3% by the method of maximum likelihood. This value significantly deviates from the expected 50% of a single-gene segregation, but is consistent with the proportion of heterozygotes for each *Idh-2*, *v* and *Est-11* loci, estimated as about 33% (Table 2). Taking these statistics into consideration we estimated the recombination percentage between the *Shd1* locus and three markers to be 9.9 ± 2.7 between *v* and *Shd1*, 11.5 ± 3.1 between *Idh-2* and *Shd1*, and 21.3 ± 3.8 between *Est-11* and *Shd1* (Fig. 3). Of the three markers, the *v* locus was nearest *Shd1*. *Idh-2*, *v*, *Shd1* and *Est-11* loci are thus arranged in this order on chromosome 2 (Fig. 3).

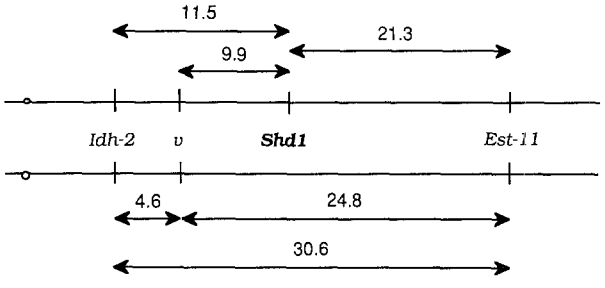

Fig. 3. A partial genetic map of four loci on chromosome 2L in barley: *Idh-2* isocitrate dehydrogenase, *v* ear type, *Shd1* a QTL for shoot differentiation, *Est-11* esterase

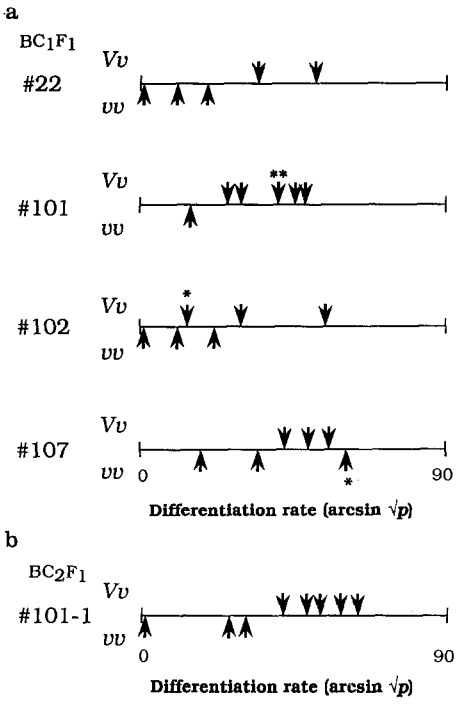

Fig. 4a, b. Relationship between ear type (*V/v*) and shoot differentiation ability. **a** For BC_1F_1 lines, backcross progeny belonging to each line was classified by heterozygous (*Vv*) and six-rowed (*vv*) types of ear. Two BC_2F_1 plants (*) were regarded as being recombinants between the *v* and *Shd1* loci. **b** A BC_2F_1 plant (**) was pollinated with 'Azumamugi' to breed BC_3F_1 plants

Genetic transfer of the Shd1K allele to the 'Azumamugi' cultivar

Four BC_1F_1 plants, nos. 22, 101, 102 and 107, all two-rowed types showing differentiation ability at 52%, 63%, 94% and 54%, respectively, were pollinated with 'Azumamugi'. Six BC_2F_1 plants from each combination (except 5 BC_2F_1 plants from no. 22) were evaluated for shoot differentiation ability with self-pollinated immature embryos. The results are summarized in Fig. 4. Two-rowed plants appeared to have higher rates of shoot differentiation than six-rowed plants. Assuming nos. 102-3 and 107-2 to be recombinants between the *v* and *Shd1* loci, we calculated the recombination frequency to be 2/23 at 8.7%, which agrees with the 9.9% estimated in the BC_1F_1 population.

A BC_2F_1 plant, no. 101-1, was further pollinated with 'Azumamugi', and the 8 BC_2F_1 plants thus produced were self-pollinated for assessment of shoot differentiation ability. As shown in Fig. 4, a closed relationship between ear type and differentiation ability was noted.

Discussion

Distorted segregation of markers

The parents, 'Kanto Nakate Gold' and 'Azumamugi', show polymorphisms for alleles not only at the *v* locus but also at the two enzyme loci, *Idh-2* and *Est-11*, on chromosome 2. However, distorted segregation was observed at these marker loci in the backcross population of this study. The BC_1F_1 population used here was obtained from a cross using a F_1 plant as the pollen donor. The distorted segregation was due to the abnormal transmission of the male gamete genotypes that participated in fertilization.

In barley, distorted segregation has been reported for alleles at the loci of waxy/non-waxy endosperm on chromosome 1 (Tabata 1961) and the esterase isozyme on chromosome 3 (Konishi et al. 1990). So far, two gametophyte factors *Ga* (Tabata 1961) and *Ga2* (Konishi et al. 1990), respectively, have been identified. A similar segregation distortion has been observed at the *v* locus on chromosome 2 (Shin et al. 1990), although no gametophyte factor has ever been identified on chromosome 2. Konishi et al. (1990) noted that the magnitude of distorted segregation was not influenced by the growing conditions of the F_1 plants, which suggests that the action of *Ga2* is stable under changing environmental conditions, whereas Schön et al. (1991) showed the distorted segregation of a certain RFLP marker (rDNA) on chromosome 7 to be the result of a temperature change at the time of pollination. The segregation distortion of *V/v* alleles in this study was not stable but environment dependent, since the degree of segregation distortion significantly varied in three experiments (Komatsuda, unpublished). The mechanism for distorted segregation at the *v* locus has still to be investigated in further detail.

Mapping of Shd1 locus

The association of differentiation ability with alleles at the *v* locus indicates that the *Shd1* locus is essential for shoot differentiation. The allele of 'Kanto Nakate Gold' designated *Shd1K* showed a significant positive effect for enhancing shoot differentiation from a callus. Enhancement of the differentiation rate is not the result of pleiotrophy of the *v* locus but the action of the *Shd1* gene, since the recombination frequency of 9.9% ± 2.7% between *v* and *Shd1* significantly differs from zero. A high percentage of shoot differentiation was observed in some six-rowed progeny, whereas no differentiation could be detected in some two-rowed progeny in anther culture-derived DH lines from 'Azumamugi' × 'Kanto Nakate Gold' (Kamatsuda, unpublished). The six-rowed cultivar 'Dissa' responds well to shoot differentiation from a callus (Lührs and Lörz 1987, 1988).

Regeneration is controlled by two major genes in alfalfa (Reisch and Bingham 1980), maize (Hodges et al. 1985) and tomato (Koornneef et al. 1987). Our study, to the best of our knowledge, is the first to map a major gene that enhances shoot differentiation. The locus of this major gene, *Shd1*, is located on a chromosome segment of approximately 25 cM situated between the *v* locus and the *Est-11* locus. *V* and *Est-11*^{Ka} alleles serve as dominant and codominant linkage markers of the *Shd1K* allele, respectively, and thus enable the selection of *Shd1K/Shd1A* heterozygotes in backcrossed progeny. The individuals used so far should be examined for differentiation ability in each generation to prevent the absence of positive alleles in other loci. It is evident that *Shd1* genes can be genetically transferred to 'Azumamugi' (difficult to differentiate) to breed a regenerable 'Azumamugi' cultivar. The "genetic transfer of genes for regeneration capacity" proposed by Kuhlmann and Foroughi-Wehr (1989) has become possible.

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