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Quantitative trait loci affecting plant regeneration from protoplasts of *Brassica oleracea*

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Abstract Quantitative trait loci (QTLs) controlling the plant-regeneration ability of *Brassica oleracea* protoplasts were mapped in a population of 128 F_2 plants derived from a cross between the high-responding, rapid-cycling line and a low-responding, broccoli breeding line of *B. oleracea*. A modified bulked segregant analysis with AFLP markers identified two QTLs for plant regeneration. In a multiple regression analysis, the two QTLs explained 83% of the total genetic variation for regeneration recorded 15 weeks after initial transfer of microcalli to regeneration medium. Both QTLs showed additive effects, and the alleles contributing to the high regeneration frequencies were derived from the high-responding, rapid-cycling line. Using microsatellites with known location, the two QTLs were mapped to linkage groups O2 and O9 on the map published by Sebastian et al. [(2000) Theor Appl Genet 100:75–81] or to chromosomes C8 and C7 on the map published by Saal et al. [(2001) Theor Appl Genet 102:695–699]. QTLs for the early flowering trait of the rapid-cycling parent have previously been mapped to the same two linkage groups. Association between flowering time and regeneration ability was, however, not found in the present material, indicating that plant-regeneration ability can be transferred between cultivars independently of the early flowering trait. The detection of two major QTLs for plant regeneration in *B. oleracea* may provide the initial step towards the identification of markers suitable for marker-assisted selection of regeneration ability.

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Introduction

Brassica oleracea contains many commercially important vegetables, including broccoli, cauliflower, cabbage and Brussels sprouts. These vegetable brassicas are becoming increasingly popular due to their nutritional value and their anti-carcinogenic properties (Beecher 1994). Although both quality and yield are currently improved through conventional breeding, further improvements of traits, such as disease resistance, delayed post-harvest senescence and yield, are limited by the availability of suitable genes in the germplasm stocks (Metz 2001; Gapper et al. 2002; Radchuk et al. 2002).

Approaches such as protoplast fusion and genetic transformation are now available for introduction of new genes into the germplasm. Somatic hybridisation has been used to transfer resistance to different diseases into *B. oleracea*, including bacterial black rot resistance from *B. napus* (Hansen and Earle 1995) and black spot resistance from *Sinapis alba* L. (Hansen and Earle 1997), *Camelina sativa* (Hansen 1998) and *Capsella bursa-pastoris* (Sigareva and Earle 1999). The 'Ogura' cytoplasmatic male sterility (CMS) system originating in *Raphanus sativus* has been improved through asymmetric protoplast fusion in *B. oleracea*, replacing *R. sativus* chloroplasts with those of *B. oleracea* (Walthers et al. 1992). Likewise, the 'Anand' CMS system originating from the wild species *B. tournefortii* has been transferred from *B. napus* to *B. oleracea* through asymmetric fusion (Cardi and Earle 1997). In addition, efficient protocols have been developed for *Agrobacterium*-mediated transformation (Metz 2001) and direct protoplast transformation (Radchuk et al. 2002). Transfer of genes for insect resistance or delayed post-harvest senescence has been achieved through genetic transformation mediated by *Agrobacterium tumefaciens* (Cao et al. 2001; Gapper et al. 2002) and *Agrobacterium rhizogenes* (Henzi et al. 2000).

A major obstacle for wide commercial application of these methods in *B. oleracea* is the lack of elite breeding lines with high capacity for plant regeneration from in vitro cultures. Thus, results mentioned above have been

achieved using *B. oleracea* lines selected for high plant-regeneration capacity. In general, the ability to regenerate shoots from in vitro plant cultures has proven to be highly dependent on both environment and genotype (Koorneef et al. 1987; Jourdan and Earle 1989; Taylor and Veilleux 1992). In *B. oleracea*, much effort has been put into improving the culture conditions for plant regeneration, but the genotype of the plant material still has a strong effect on regeneration of *B. oleracea* cell culture systems (Jourdan and Earle 1989; Loudon et al. 1989; Zhao et al. 1995).

The genetic nature of regeneration has been studied in tissue culture systems of various species through Mendelian and quantitative genetic studies. Results from these studies have indicated that the trait is controlled by a limited number of genes in the species investigated (Koorneef et al. 1987; Nadolska-Orczyk and Malepszy 1989; Armstrong et al. 1992; Taylor and Veilleux 1992; Hansen et al. 1999). Recently, QTL analysis of plant regeneration has been used to obtain more precise information about number of genes, their chromosomal location, significance and associated markers in a number of species including sunflower (Flores Berrios et al. 2000), barley (Komatsuda et al. 1995; Mano et al. 1996), tomato (Koorneef et al. 1993), maize (Armstrong et al. 1992), rice (Taguchi-Shiobara et al. 1997), wheat (Ben Amer et al. 1997) and *Arabidopsis thaliana* (Schiantarelli et al. 2001).

In *B. oleracea*, a genetic study of plant regeneration from protoplast-derived microcalli of F_2 progenies from a cross between two parents with high (rapid-cycling line of *B. oleracea*) and low (elite broccoli line) plant-regeneration ability was previously published (Hansen et al. 1999). Results indicated that the regeneration ability from microcalli of *B. oleracea* protoplasts is controlled by two to three genes. In the present study, this plant material and the corresponding evaluation data were used to identify and locate major QTLs contributing to the plant-regeneration ability of protoplast-derived microcalli. In wheat, a connection between early flowering time and high plant-regeneration ability has previously been suggested (Ben Amer et al. 1996, 1997). Since the rapid-cycling line used as the high-responding parent of the mapping population also possesses the early flowering trait, the possible relationship between these two traits was also investigated.

Materials and methods

Plant material and protoplast culture

The F_2 plants used for QTL mapping were obtained by selfing a single F_1 hybrid plant derived from a cross between an elite broccoli breeding line (360-7) from Daehnfelt (Denmark) and rapid cycling (RC) *B. oleracea* (Crucifer Genetics Cooperative no. 3–1, Williams and Hill 1986) with low and high ability to regenerate shoots from protoplast-derived microcalli, respectively (Hansen et al. 1999). Nine plants of the RC *B. oleracea* parent (P_1), ten plants of the broccoli parent (P_2) and 248 individual F_2 plants were evaluated for plant regeneration from mesophyll protoplasts according to methods described previously (Hansen et al 1999). At least 100 protoplast-derived calli were transferred to regeneration medium for each F_2 plant. Regeneration frequencies were recorded 5, 10 and 15 weeks after initial transfer of individual microcalli to regeneration medium. Regeneration was estimated as the percentage of calli with shoot initiations of at least 0.5 cm in length (Hansen et al. 1999).

The mapping population consisted of selfed seed offspring from the 20 F_2 plants showing the highest, and the 20 F_2 plants showing the lowest, regeneration frequencies, together with 88 F_2 plants randomly selected from the remaining 208 F_2 plants.

For registration of flowering time, approximately 20 seeds of each of the 128 F_2 plants used for QTL analysis of plant regeneration were germinated, and the flowering status of each F_3 plant was measured once every week. The final flowering time of the F_2 plants was estimated as the number of days from sowing to the flowering of half of the F_3 plants.

Genetic marker analysis

Seeds from the self-fertilised parents and F_2 plants were germinated, and DNA was extracted from fresh leaf material using the phenol-chloroform protocol described by Sharp et al. (1988). DNA was extracted from a mixture of at least 20 different F_3 plants derived from each F_2 parent in order to represent the segregation of loci in the original F_2 plants.

AFLP analysis was essentially carried out according to the protocol described by Vos et al. (1995). A total amount of 300 ng DNA was digested with 1.5 U *Eco*RI (MBI Fermentas) and 1.5 U *Mse*I (New England Biolabs). Restriction fragments were ligated with complementary doubled-stranded adaptors, and the adaptor-ligated fragments were used as templates for preamplification with *Eco*RI+1 and *Mse*I+1 primers (one selective nucleotide). The PCR mixture for preamplification contained 1× reaction buffer (HT Biotechnology, England), 0.1 mM of each dNTP, 22.5 ng *Eco*RI+1 primer, 22.5 ng *Mse*I+1 primer, 2.0 µl ligated DNA and 0.25 U of Super-*Taq* polymerase (HT Biotechnology). Reactions took place in 0.6-ml PCR tubes with final volumes of 15 µl. The temperature profile was as described by Vos et al. (1995), and reactions were amplified on a Thermocycler (Thermo Hybraid HB-MBS-0.5). Diluted preamplification mixtures (1:25) were used as templates for the selective amplification with *Eco*RI+2 primers combined with *Mse*I+2 or *Mse*I+3 primers. PCR reactions for selective amplification were performed in 96-well microtitre plates with final reaction volumes of 10 µl. PCR mixtures for selective amplification contained 1× reaction buffer (HT Biotechnology), 0.1 mM of each dNTP, 12.5 ng *Eco*RI primer, 15 ng *Mse*I primer, 2.5 µl diluted preamplification mix and 0.2 U of Super-*Taq* polymerase (HT Biotechnology). The temperature profile of selective amplification and protocols used for electrophoresis and silver staining of the gels have been described previously (Torp et al. 2001). Silver-stained gels were air-dried and scanned using an Epson Perfection 1650 scanner. The molecular weight marker Lamda DNA/Eco471 (MBI Fermentas) was included on each gel for size determination. Lengths of polymorphic amplification products were determined using the software RFLPscan Plus 3.12 of the software package Gene ImagIR version 4 (LI-COR). Polymorphic AFLP markers were named by the *Eco*RI/*Mse*I primer pair combination followed by the length of the amplification product in base pairs.

A modified bulked segregant analysis (Michelmore et al. 1991) was used to focus polymorphic AFLP markers for the QTL analysis. AFLP primer combinations showing polymorphisms between the parents were tested on the 20 F_2 offspring showing highest, and the 20 F_2 offspring showing lowest, plant-regeneration capacity after 15 weeks of regeneration. Significant association between the polymorphic markers and the regeneration data for these 40 extreme plants was tested with simple analysis of variance using PROC GLM of the software package SAS (SAS institute 1989). Subsequently, AFLP primer combinations showing one or more significant associations with plant regeneration among the 40 extreme plants were scored in the remaining 88 plants of the

mapping population. Most of the selected AFLP primer combinations also identified additional non-significant polymorphisms, which were scored and included in the data set.

Twenty microsatellites with known location on the *B. oleracea* chromosomes were used to assign the AFLP markers to linkage groups of previously published maps. Three of these microsatellites are described on the Web site http://www.hri.ac.uk/uk-brc/kingbrassica/pubssra.htm and incorporated into the integrated map of Bohuon et al. (1996, 1998) and Sebastian et al. (2000). The remaining 17 microsatellites have been described by Saal et al. (2001) and incorporated into the map of Hu et al. (1998). PCR reactions for microsatellite analysis were carried out as described by Torp et al. (2001). Electrophoresis, silver staining and size determination were as described above for AFLP analysis, except that 6% polyacrylamide gels were used for separation of PCR products.

Map construction and QTL analysis

A focussed map of AFLP markers was constructed using JoinMap 2.0 software (Stam and Van Ooijen 1995). An LOD score of 5.5 was used for initial grouping of markers. Calculation of map distances was done using the Kosambi mapping function (Kosambi 1944). Markers showing significantly distorted segregation were discarded before map construction.

For QTL mapping, regeneration frequencies were arcsin square root transformed to improve normal distribution and the transformed data were used for simple interval mapping with the software package PlabQTL (Utz and Melchinger 1995). An empirical LOD value corresponding to 5% LOD threshold was estimated from 2,000 permutations of the data set. For significant QTLs, PlabQTL estimated support intervals with an LOD fall off of 1.0.

Broad-sense heritability of the trait (h^2) was estimated from the average variance of the arscin square root-transformed data of

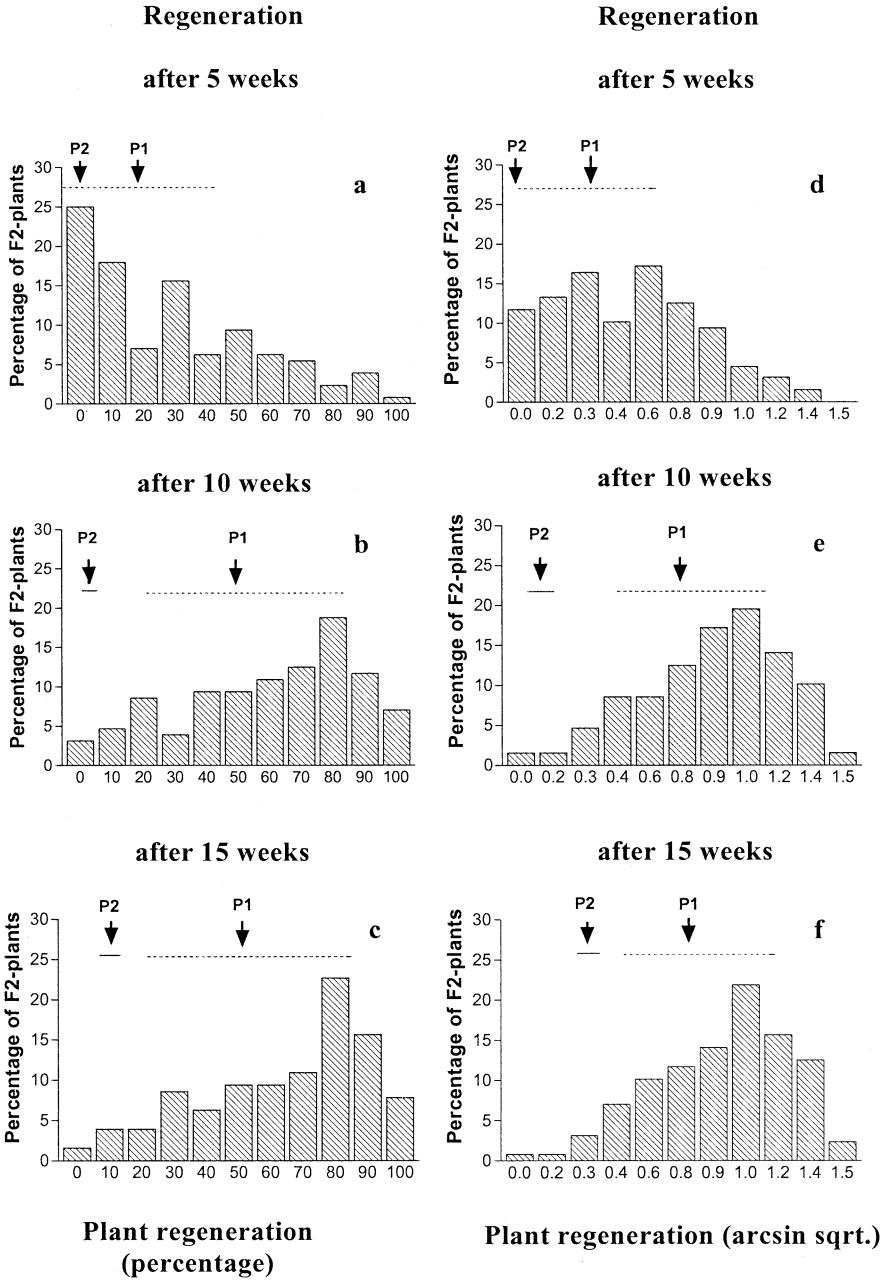

Fig. 1 Percent distribution of regeneration percentage among 128 F_2 plants derived from the F_1 hybrid between P_1 rapid-cycling (RC) *B. oleracea* and P_2 broccoli line (*B. oleracea*) after **a** 5 weeks on regeneration media, **b** 10 weeks on regeneration media and **c** 15 weeks on regeneration media. Arcsin-transformed regeneration data are shown in **d**, **e** and **f** after 5, 10, and 15 weeks on regeneration media, respectively. *Arrows* indicate the average parental regenerations. The *black lines* represent the standard error of P_1 means and the *dotted lines* represent the standard error of P_2 means

parent lines (V_e) and among the 128 F_2 offsprings (V_p). The genetic variance (V_g) was estimated as $V_p - V_e$, and broad-sense heritability was estimated as $h^2 = V_g/V_p$. The percentage of phenotypic variation explained by the two QTLs was estimated by PlabQTL ($R^2\%$), and the percentage of genetic variation explained by the two QTLs as $R^2\%/h^2$. Predicted values of regeneration frequency of the two parents were calculated from the PlabQTL-estimated values of means and additive effects of the individual QTLs.

Flowering-time data were tested for possible correlation with plant-regeneration ability by a simple correlation analysis using PROC CORR (SAS Institute 1989). Furthermore, possible linkage between QTLs for flowering time and plant regeneration was tested by simple interval mapping in PlabQTL (Utz and Melchinger 1995), using the focussed linkage map constructed in this study for the QTL analysis of plant regeneration.

Results

Plant-regeneration frequencies from protoplast cultures of the two parents were clearly different for all three data sets recorded 5, 10 and 15 weeks after transfer of microcalli to regeneration media, respectively (Fig. 1a–c). After 5 weeks on regeneration media, the high-responding parent showed an average plant-regeneration frequency of 16.9±25.1%, while none of the microcalli from the low-responding parent regenerated plants. Average regeneration frequency of protoplast-derived colonies from the 128 F_2 plants was 28.2±26.4%, with many F_2 offspring plants showing higher plant-regeneration frequencies than the high-responding parent. Protoplast plant-regeneration frequencies of both parents and F_2 plants increased with time. Ten weeks after transfer of colonies to regeneration medium, the high-responding parent showed a regeneration frequency of 50.1±32.1%, while the regeneration frequency of the low-responding parent had increased to 1.7±1.5%, and average regeneration frequency from all F_2 plants was 59.3±27.2% (Fig. 1b). Fifteen weeks after transfer to regeneration medium, average regeneration frequencies of 52.9±32.0% and 9.4±3.2% were observed for the high- and low-responding parent, respectively, while average regeneration from all F_2 plants had increased to 63.6±26.1%. Still, many of the F_2 offspring plants showed higher plant-regeneration frequencies than the high-responding parent (Fig. 1c). The data were arcsin square root transformed in order to improve normal distribution of the data and to reduce the effect of the percentage scale on sampling variance (Fig. 1d–f). This transformation improved fit of models and was used for all subsequent analysis of the data.

In the present study, we focussed our QTL analysis on chromosomal regions with markers, which showed significant association with regeneration frequencies among the 20 F_2 offspring from each of the two extremes of the regeneration distribution recorded 15 weeks after initiation of regeneration. Two hundred and fifty AFLP markers from 35 different primer combinations were analysed with the 20 highest and 20 lowest plant-regenerating F_2 offspring. Among these 250 AFLPs, 26 markers from 16 different primer combinations showed significant association with regeneration frequency of the extreme lines and were scored for segregation with all 128 F_2 offspring. Most of the primer combinations with one or more significant associations between a marker and the trait identified additional non-associated polymorphisms, which were also included in the data set. Hence, a total of 72 AFLP markers were scored in the entire F_2 population. In addition, 20 microsatellite markers located on different linkage groups were scored for all 128 F_2 offspring and included in construction of the focussed map. Six of these microsatellites showed significant associations with regeneration frequency in

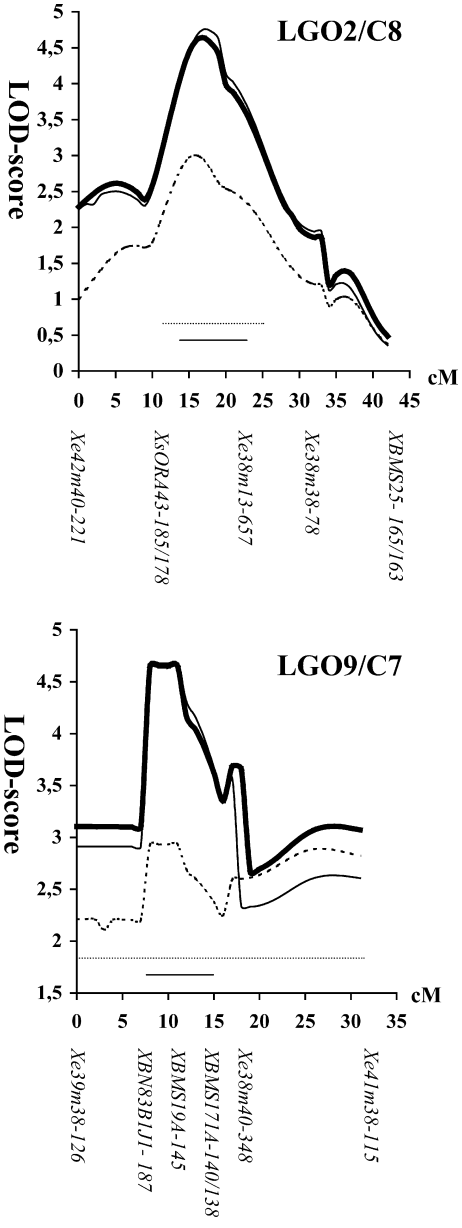

Fig. 2 QTL likelihood maps for protoplast regeneration on linkage groups O2/C8 and O9/C7 for recordings after 5 weeks (*dotted lines*), 10 weeks (*thick black lines*) and 15 weeks (*thin black lines*). *Dotted lines* represent the one LOD support interval estimated by PlabQTL for the recording after 5 weeks, and *black lines* represent the one LOD support interval for recordings after 10 and 15 weeks

Table 1 Position and effect (±SE) of the QTLs at linkage group O2/C8 and O9/C7 for the three regeneration recordings in the F_2 population (arcsin square root-transformed scale)

QTL	Regeneration after	Position	LOD score	Permutation 5% LOD threshold	One LOD interval	Additive effect[a]	Dominant effect[a]
QTL at linkage group O2/C8	5 weeks	16	3.00	3.09	10–26	0.128±0.045**	–0.182±0.073*
	10 weeks	17	4.64	3.09	12–23	0.167±0.039**	–0.124±0.063
	15 weeks	17	4.75	3.16	13–23	0.164±0.037**	–0.112±0.060
QTL at linkage group O9/C7	5 weeks	11	2.93	3.09	0–31	0.152±0.041**	–0.007±0.063
	10 weeks	11	4.65	3.09	7–15	0.163±0.036**	–0.060±0.055
	15 weeks	11	4.68	3.16	7–15	0.153±0.034**	–0.072±0.052

[a] A positive value means that the allele from P_1 (rapid cycling, *B. oleracea*) increases the trait
Significance levels: *P=0.05, **P=0.01

Table 2 Variance (V), broad-sense heritability (h^2) and percentage of variation (±SE) explained by the two QTLs for the three regeneration recordings (arcsin square root-transformed scale)

Regeneration after	P_1 (V_{P1})	P_2 (V_{P2})	Environment (V_e)	F_2 (V_p)	h^2	Variance explained by QTLs (%)	
						Phenotypic	Genotypic
5 weeks	0.1089	0.0000	0.0545	0.1205	0.548	19.3±6.3	35.2 ±11.5
10 weeks	0.1225	0.0049	0.0637	0.1049	0.392	28.6±6.7	73.0±17.1
15 weeks	0.1225	0.0025	0.625	0.0959	0.348	28.8±6.8	82.8±19.5

Table 3 Predicted and observed values (±SE) for protoplast regeneration of the two parents after 5, 10 and 15 weeks of regeneration

	Regeneration after		
	5 weeks (%)	10 weeks (%)	15 week (%)
P_1 (PlabQTL estimated)	39.9±0.3	81.5±0.2	84.4±0.2
P_2 (PlabQTL estimated)	1.5±0.3	20.2±0.2	25.6±0.2
P_1 (observed)	16.9±25.1	50.1±32.1	52.9±32.0
P_2 (observed)	0.0	1.7±1.5	9.4±3.2

the extreme line analysis. The partially focussed map created by JoinMap grouped 44 of the AFLP markers and ten microsatellites in seven linkage groups. This map included 23 AFLP markers and five microsatellite markers showing significant association with plant regeneration from extreme line analysis.

Simple interval mapping with PlabQTL identified two QTLs affecting protoplast regeneration frequency. Comparable QTL maps for all three recordings were obtained for both QTLs (Fig. 2, Table 1). For both QTLs, the maximum LOD scores for the regeneration recorded after 10 and 15 weeks were almost identical and significant at the 5% level based on analysis of 2,000 permutated data sets. Only for regeneration after 5 weeks were the maximum LOD-scores were too low to be significant (Table 1). Apparently, shoot initiation was not completed in many of the microcalli after 5 weeks of the regeneration process in this study. Since large increases in regeneration frequencies were no longer observed from 10 to 15 weeks, the recordings at 15 weeks seem to be the most accurate measurements for regeneration capacity. Thus, only QTL results obtained for regeneration frequencies recorded 15 weeks after regeneration initiation will be further discussed. Among the 24 markers included in the two linkage groups with QTLs, 22 markers were among those initially selected for significant association with regeneration frequency during extreme line analysis. Before final QTL analysis, the number of markers in the two chromosomal regions with QTLs was reduced due to very close linkage between several of them (Fig. 2). Both of the QTLs could be assigned to specific linkage groups of previously published maps using microsatellites with known location. One QTL was located on linkage group O2 on the map published by Sebastian et al. (2000) and on linkage group C8 on the map published by Saal et al. (2001). The other QTL was located on linkage group O9 and linkage group C7 on the maps published by Sebastian et al. (2000) and by Saal et al. (2001), respectively.

Estimated additive effects were significant for both QTLs, and the alleles contributing to high-regeneration frequencies were, in both cases, derived from the high-responding parent, rapid-cycling *B. oleracea*. No significantly dominant effects of the QTLs were observed after 15 weeks (Table 1). A simultaneous multiple regression with both QTLs could explain 28.8% of the phenotypic variation for frequency of plant regeneration (Table 2). Broad-sense heritability values were estimated from the F_2 plants used as the mapping population in this study. These values were used to calculate the percentage of genotypic variation explained by the two QTLs found in this study. The percentage of the genotypic variation explained by the two QTLs was estimated to be 82.8%

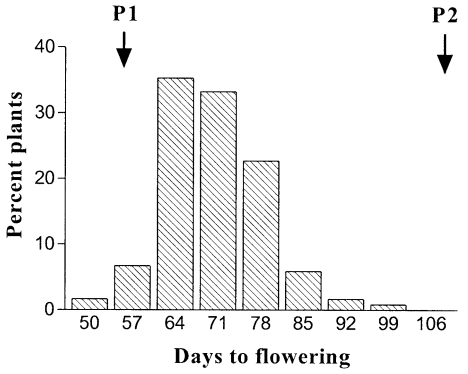

Fig. 3 Distribution of flowering time among the F_2 plants. The parental means are indicated with *arrows*

after 15 weeks of regeneration (Table 2). Estimated means and additive effects from the fitted models were used to calculate predicted values for protoplast plant regeneration of the parents of the mapping population (Table 3). The predicted regeneration values were higher than the observed values for both parents. For regeneration recorded after 15 weeks, the predicted value for the high-responding parent (P_1) was 84.4±0.2%, while the corresponding observed value for P_1 was 52.9±32.0%. The predicted value for the low-responding parent (P_2) was 25.6±0.2% for recordings after 15 weeks, while the corresponding observed value for P_2 was 9.4±3.2%. However, when the high standard errors of observed regeneration frequencies were considered, the correspondence between the two values was relatively good.

Distribution of flowering time among plants of the mapping population is shown in Fig. 3. No correlation was found between the flowering-time data and the plant-regeneration data for any of the three recordings (P=0.62, P=0.30 and P=0.38 for the recordings 5, 10 and 15 weeks after initiation, respectively). In addition, QTL analysis with the focussed linkage map constructed for this study showed no significant QTLs for flowering time on the partial map.

Discussion

The focussed mapping approach used in this study proved to be a very fast and efficient method to identify QTLs. Only a partial map covering approximately 30% of the total *B. oleracea* genome was constructed by this procedure, but the QTL-likelihood maps of the two QTLs identified included 69% of the markers initially showing significant association with plant regeneration in extreme line analysis. The two QTLs both with additive effects from the high-responding parent proved to be major QTLs explaining 83% of the total genetic variation for regeneration. The identification of these two major QTLs affecting the trait is in good agreement with conclusions from our previous semi-qualitative analysis of the data (Hansen et al. 1999). The previous study proposed two to three genes with mainly dominant effects. The more sensitive marker-based analysis used in this study has, however, reduced the number of affecting genes to two and rejected major dominant effects.

Similar QTL studies of protoplast plant regeneration have not previously been reported in *Brassica* or in other species. Identification of two major QTLs with additive gene effects, however, is in accordance with several studies conducted with other types of somatic cell culture systems in other species. These studies have revealed a low number of genes with mainly additive effects, explaining a large proportion of the genetic variation associated with plant regeneration from in vitro cultured cells (Armstrong et al. 1992; Koorneef et al. 1993; Komatsuda et al. 1995; Taguchi-Shiobara et al. 1997). In the brassica-model plant *A. thaliana*, QTL analysis of plant regeneration from root and leaf explants was reported by Schiantarelli et al. (2001). QTLs were identified on chromosome 1, 4 and 5 for total number of regenerated shoots from leaf explants and on chromosome 5 for total number of regenerated shoots from root explants. The QTLs identified on *A. thaliana* chromosome 5 are located approximately 40 cM from the top of this chromosome. Interestingly, the linkage groups O2 and O9 of *B. oleracea* harbouring the QTLs found in this study both contain regions of 50 cM to 70 cM, which have been reported to be homoeologous copies of a region from the top of *A. thaliana* chromosome 5 (Bohuon et al. 1998). The QTLs for regeneration found on *A. thaliana* chromosome 5 might therefore be homoeologous to one or both QTLs identified for regeneration in *B. oleracea*. Common chromosomal regions with QTLs for regeneration from different types of tissue akin to those observed in *A. thaliana* for leaf and root tissue may indicate the presence of a common set of genes affecting plant regeneration from any somatic tissue. The rapid-cycling *B. oleracea* line used as the parent with high protoplast plant-regeneration ability in this study also shows good regeneration ability in other types of cell culture systems including *Agrobacterium*-transformed tissue (Berthomieu et al. 1994; Christey et al. 1997) and direct shoot regeneration from stem explants (Cheng et al. 2001). Development of protoplasts into plants passes over an intermediate callus phase from which shoots are regenerated. It is therefore likely that genes controlling plant regeneration from protoplasts are also involved in regeneration from callus or cells derived from other types of somatic tissue cultures.

The regions on linkage groups O2 and O9 of *B. oleracea*, which contain homoeologous copies of an area of *A. thaliana* chromosome 5, also contain QTLs affecting flowering time in *B. oleracea* as well as in *A. thaliana* (Bohuon et al. 1998). Previously, a possible connection between plant regeneration and genes regulating flowering time in wheat was suggested by Ben Amer et al. (1996, 1997). They found a major QTL for wheat callus regeneration located on chromosome 2B close to where a gene for 'earliness per se' has been mapped. This gene modifies ear emergence time in wheat by regulating the

number and rate of primordial initiations (Worland 1996). Although the rapid cycling line used as the high-regeneration parent of the mapping population also possesses the early flowering trait, such a relationship does not seem to exist in the present material of *B. oleracea*. Neither QTL analysis nor simple correlation analysis indicated association between earliness and plant regeneration.

Several genes thought to be involved in shoot regeneration have now been identified in *A. thaliana*, including the *SRD* (*shoot redifferentiation*) genes (Yasutani et al. 1994), the *STM* (*shoot meristemless*) gene (Barton and Poethig 1993), the *IRE* (*increased organ regeneration*) gene (Cary et al. 2001), the *HOC* (*high organ capacity*) gene (Catterou et al. 2002) and the *ESR1* (*enhancer of shoot regeneration*) gene (Banno et al. 2001). These genes or other genes with a shoot-regulating effect on shoot regeneration may be candidate genes for the QTLs identified for plant regeneration ability in *B. oleracea*. Although the locations of most of these genes on the *A. thaliana* chromosomes are known, it is not yet possible to predict locations of these *A. thaliana* genes on *B. oleracea* maps. Progress in basic *Brassica* genome programmes may, however, make this possible in the near future, and this may help elucidating the function of these major QTLs in the shoot regeneration process.

Different studies have shown that large improvements in regeneration ability can be obtained through introgression of major high-responding genes into recalcitrant lines (Armstrong et al. 1992; Koorneef et al. 1993; Komatsuda et al. 1995). Such backcrossing of genes for regeneration is highly facilitated if markers linked to the genes can be used for selection. In the present study, codominant microsatellite markers closely linked to the QTLs were identified on both linkage groups. However, the suitability of these microsatellite markers for selection has to be assessed through evaluations of the co-segregation between the markers and the regeneration ability in different *B. oleracea* populations. Alternatively, further fine mapping of the two QTLs may be needed to identify more tightly linked markers able to facilitate the rapid transfer of high-regeneration ability into recalcitrant, elite breeding lines.

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