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QTL analysis of potato tuberization

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Abstract Quantitative trait loci (QTLs) affecting tuberization were detected in reciprocal backcrosses between *Solanum tuberosum* and *S. berthaultii*. Linkage analyses were performed between traits and RFLP alleles segregating from both the hybrid and the recurrent parent using a set of framework markers from the potato map. Eleven distinct loci on seven chromosomes were associated with variation in tuberization. Most of the loci had small effects, but a QTL explaining 27% of the variance was found on chromosome 5. More QTLs were detected while following alleles segregating from the recurrent *S. tuberosum* parent used to make the backcross than were detected by following alleles segregating from the hybrid parent. More than half of the alleles favoring tuberization were at least partly dominant. Tuberization was favored by an allele from *S. berthaultii* at 3 of the 5 QTLs detected by segregation from the hybrid parent. The additive effects of the QTLs for tuberization explained up to 53% of the phenotypic variance, and inclusion of epistatic effects increased this figure to 60%. The most common form of epistasis was that in which presence of an allele at each of 2 loci favoring tuberization was no more effective than the presence of a favorable allele at 1 of the 2 loci. The QTLs detected for tuberization traits are discussed in relationship to those previously detected for trichome-mediated insect resistance derived from the unadapted wild species.

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Introduction

The ability to tuberize is under the control of environmental factors, but within the genus *Solanum* there is great genetic variability in the tuberization response to a given environment. This can lead to poor tuberization of hybrid progenies in temperate environments when wild *Solanum* species are used for breeding. For example, the wild Bolivian species, *S. berthaultii*, carries trichome-mediated insect resistance but is very late maturing in northern climates because it requires short days for tuberization. When breeders have tried to introgress the insect resistance into cultivated potatoes, the presence of desirable type-B trichome "droplets" has been difficult to separate from the donor's inability to tuberize under long days (Kalazich and Plaisted 1991). If this difficulty is caused by linkage of the traits, a possible solution to the problem would be breeding based upon genotype rather than phenotype. The association of molecular markers with the desired traits would make such an approach possible.

Bonierbale et al. (1994) reported the construction of two linkage maps from restriction fragment length polymorphism (RFLP) segregation in the backcrosses of *(Solanum tuberosum*×*S. berthaultii)*×*S. tuberosum*, and of *(Solanum tuberosum*×*S. berthaultii)*×*S. berthaultii*. They described the segregation for trichome characteristics that are related to insect resistance in these populations. Quantitative trait locus (QTL) analysis revealed 6 loci affecting type-A trichome characteristics and 5 affecting type-B trichome characteristics (Bonierbale et al. 1994). In the present paper we report RFLP linkage analyses of traits related to tuberization in the same two backcross populations. To describe the status of the potato plant when it becomes capable of forming tubers, it is common to say that the plant has become induced to tuberize. Induction to tuberize is favored by cool temperatures and a number of other envi-

Fig. 1 Apical cuttings after 14 days, illustrating genotypic differences in ability to tuberize as used to describe the Cutting-rating (year 1) and Cutting-rating (year 2) traits. Cuttings from *left* to *right* show progressively stronger tuberization responses at the buried basal bud: no growth, orthotropic shoot, diagravitropic stolon, stolon terminated by tuber, and sessile tuber. The first cutting represents a "1" on our rating scale; the last represents a "9." One-node cuttings give the same responses

ronmental conditions (Ewing and Struik 1992), but the most important factor is the exposure of leaves to long nights. Red light interruptions of the dark period interfere with induction, an effect that is reversible by subsequent exposure to far-red light (Batutis and Ewing 1982). Although all genotypes seem to become more strongly induced as photoperiods are shortened, some can become induced even under continuous light, while others require photoperiods of 12 h or less (Ewing and Struik 1992); i.e., some have much longer critical photoperiods than others (Ewing 1978; Ewing and Wareing 1978). The shorter the critical photoperiod of a genotype, the later is its time of tuberization and its maturity under temperate field conditions. From heritability studies, Mendoza and Haynes (1977) hypothesized that the ability to tuberize under long photoperiods is controlled by a few major, recessive genes and a number of minor genes.

There is a continuum of responses to photoperiod that reflects the relative intensity of the tuber-inducing stimulus. Non-inducing conditions produce neither stolons nor tubers; weak induction promotes stolon initiation; stronger induction leads to tuber formation on the stolons; and very strong induction favors direct tuber formation in the absence of stolons (Ewing and Struik 1992). These responses are seen on whole plants and are conveniently observed in stem cuttings. The growth response at the buried buds of cuttings (Fig. 1) is highly correlated with the mother plant's earliness to tuberize (Rasco et al. 1980; Ewing 1985; Furumoto et al. 1991). In our studies we grew plants under conditions only moderately favorable for induction. We then used cuttings and early tuberization on whole plants to detect QTLs affecting the ability to tuberize under long photoperiods, hence QTLs affecting maturity.

We found 11 distinct QTLs affecting tuberization. We discuss gene action at the QTLs, and we give an explanation of the association between functional type-B trichomes and late tuberization.

Materials and methods

Plant material and conditions for induction of tuberization

The dihaploid *S. tuberosum* USW2230 (a haploid from cv 'Saco') and the diploid *S. berthaultii* (PI 473331) were crossed. One of the resulting hybrids was crossed as a female to a haploid *S. tuberosum* (HH1-9) to create a backcross to *S. tuberosum* (BCT). The same hybrid clone was crossed to another seedling of the *S. berthaultii* accession to create a backcross to *S. berthaultii* (BCB) (Bonierbale et al. 1994). Progenies of both backcrosses were maintained *in vitro*.

Five tissue culture plantlets (Espinoza et al. 1986) of each of 288 BCB and 299 BCT clones were grown in clay pots filled with a peat medium (Boodley and Sheldrake 1982) in a polyethylene-covered greenhouse in Ithaca, New York. The experiments were conducted during the months of June to September. The light intensity varied from about 250 $\mu E\ m^{-2}\ s^{-1}$ on a cloudy day to about 1500 $\mu E\ m^{-2}\ s^{-1}$ on a sunny day. Day temperatures varied between 15°C and 35°C, and night temperatures between 15°C and 20°C. The natural photoperiod (> 5 footcandles) at Ithaca is 15.7 h on June 1, 15.9 h on July 1, 15.0 h on August 1, and 13.6 h on September 1 (Francis 1970). Photoperiods of 10 h were provided by covering the plants with black cloth at 1830 h and withdrawing the cloth at 0830 h. Plants of each family were randomized within five blocks in the greenhouse. To assure that at least one of the five blocks would receive photoperiods that would differentiate tuberization response among genotypes, we treated each block with a different number of short days or harvested at a different plant age (Table 1).

Assessment of earliness by examination of cuttings

Following the short-day treatments three cuttings were taken from each plant for evaluation of tuber induction, as described by Ewing (1985). In brief, nodal cuttings consisting of leaves and their subtended buds were taken from the fifth, sixth, and seventh nodes below the apex (node 1 being the youngest node with a leaf longer than 35 mm). The buds of the cuttings were inserted into peat medium, and the cuttings were placed in a horticultural mistbench with continuous light at 15 $\mu E\ m^{-2} s^{-1}$. After 2 weeks in the mistbench, the buried bud of each cutting was rated for extent of tuber induction. Figure 1 illustrates the range in response. A higher rating on the scale of 1–9 indicates a stronger level of tuber induction and hence an earlier maturity (Ewing and Struik 1992; Fig. 1). The mean rating (five blocks, three cuttings per block) for tuber-induction measured on cuttings is referred to as "Cutting-rating (year 1)". Values for missing cuttings were estimated according to the usual missing data formula for randomized block experiments (Snedecor and Cochran 1980).

Table 1 Number of days from planting until cutting and number of 10-h photoperiods in the five blocks of plants of 299 genotypes from BCT and 288 genotypes from BCB in 1990. The percentage of tuberized plants was recorded at cutting

BCT			BCB		
Block no.	Number of days from planting until cutting	Number of 10-h days	Block no.	Number of days from planting until cutting	Number of 10-h days
1	58	0	1	51	0
2	51	0	2	35	5
3	45	3	3	43	9
4	42	5	4	48	11
5	34	7	5	34	7

Table 2 Number of days from planting until cutting and number of 10-h photoperiods in the 1991 experiments. There were four blocks of 158 genotypes from BCT and 17 genotypes from BCB

Block no.	Number of days from planting until cutting	Number of 10-h days
1	34	5
2	42	3
3	30	3
4	37	7

Table 3 Brief descriptions of traits. For further details, see text

Cutting-rating (year 1):	Mean rating of one-node cuttings in 1990; average of five blocks, three cuttings per plant
Cutting-rating (year 2):	Mean rating of one-node cuttings in 1991; average of four blocks, one cutting per plant
%Tuberized plants:	Mean percentage of plants that had tuberized in 1990 by the time that cuttings were taken, blocks two through five (Table 1)

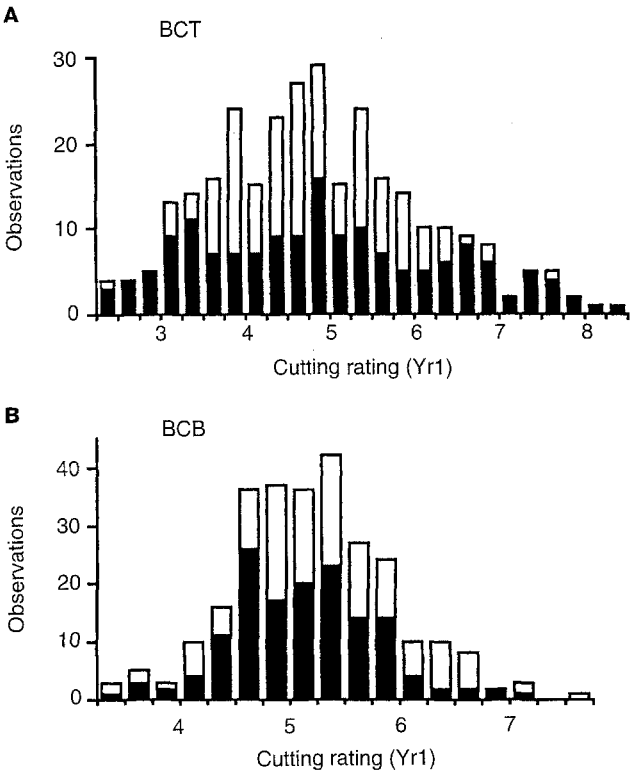

Fig. 2A, B Distribution of Cutting-rating (year 1) data. The *total length* of each *bar* represents the entire population; the *filled portion* represents the individuals taken for RFLP genotyping. **A** BCT; **B** BCB

Assessment of earliness by examination of whole plants

After cuttings were taken, plants in blocks two through five (Table 1) of each backcross family were harvested to assess early tuberization on the whole plant. (Plants in block one of each family were saved for final tuber yields.) The percentage of plants of a particular genotype that had tubers at the time cuttings were taken constituted the trait "% Tuberized plants". The data were transformed by arcsin to improve normality for analysis, and the means were then transformed back for presentation.

Repetition of the tuberization assays on a subset of the plants

These assays were repeated in 1991 on subsets of each family. Four plants each of the 155 BCT clones selected for genotyping (see later), the 17 clones representative of the BCB family, and the 25 interspecific hybrid clones (USW2230×PI473331) were grown under the same greenhouse conditions as in 1990. Four blocks were treated with discrete numbers of short days, and cuttings were taken at different plant ages (Table 2). A one-node cutting consisting of node 5 and its sub-tended leaf was taken from each plant. Rating of the underground buds on the 1–9 scale (see above) resulted in data for the trait "Cutting-rating (year 2)". Tuberization on apical and two-node cuttings (Ewing 1985) and tuber fresh weight on whole plants were also determined. The traits Cutting-rating (year 1), Cutting-rating (year 2), and % Tuberized plants are summarized in Table 3.

Selective genotyping

As explained previously (Bonierbale et al. 1994) we used a modified form of selective genotyping, selecting half of the 300 individuals from each backcross family to include the extremes of the phenotypic distributions for several traits in RFLP analysis. The maximum likelihood methods used by the MAPMAKER-QTL software (Lincoln and Lander 1989) allowed selective genotyping without biasing the outcome, as long as the phenotypic data of the entire population were included in the dataset (Lander and Botstein 1989). In BCB the selection of 150 seedlings for genotyping was based on trichome characteristics (Bonierbale et al. 1994). Selection of the 155 seedlings to be used for genotyping in BCT was based on Cutting-rating (year 1),% Tuberized plants, and tuber dormancy characteristics (Van den Berg 1993). The effect of the selection procedure on the frequency distributions of Cutting-rating (year 1) is presented in Fig. 2.

Linkage analyses

The genotypic data used for this paper are described in detail by Bonierbale et al. (1994). In the present analyses, alleles (defined as restriction fragments segregating at particular marker loci) were monitored for each parental source (hybrid and recurrent) in each of the two backcross families (BCT and BCB). Four data sets were therefore available as follows: In BCT, (1) *S. berthaultii* alleles (B) from the hybrid parent segregating in the recurrent *S. tuberosum* background, and (2) *S. tuberosum* alleles (T^R) segregating from the recurrent parent; in BCB, (3) *S. tuberosum* (T) alleles from the hybrid parent segregating in the recurrent *S. berthaultii* background, and (4) *S. berthaultii* alleles (B^R) segregating from the recurrent parent. Dataset 3 corresponds to the segregation on which the comparative tomato-potato maps were based (Tanksley et al. 1992), and both sets 1 and 3 were analyzed previously for correlations with trichome-mediated insect resistance (Bonierbale et al. 1994).

Interval mapping was performed with MAPMAKER QTL (Lincoln and Lander 1989) for datasets 1 and 3, which covered the genome with markers approximately every 10 cM. Because the number and distribution of markers segregating from the recurrent parents (datasets 2 and 4) did not cover the genome homogeneously, analysis was performed for individual marker-trait associations by one-way ANOVA, regarding marker classes as treatments (Proc GLM; SAS Institute 1985).

As the significance levels of QTLs found by ANOVA could possibly have been affected by the selective genotyping, a P value of 0.01 (-LOG [P value]=2) was chosen for the threshold significance level. We also present phenotypic means for RFLP genotypes at QTLs. A LOD score of 2.5 was used as the significance threshold for QTLs detected using interval mapping. The interaction term from two-way ANOVA tests, calculated with SuperANOVA software (Abacus Concepts 1989), was used to assess epistasis. In order to make a broad search for epistasis, we performed such analyses among the marker loci linked to QTLs that had LOD>1 or -(LOG [P])-values>1, including marker loci associated with tuberization traits not presented in this paper (Van den Berg 1993).

About one-fourth of the plants in BCT showed reduced growth and vigor associated with small and chlorotic leaves. Tests revealed that this was not related to a virus, but to a genetic defect. The chlorosis trait mapped to a single region on chromosome 1 (Van den Berg 1993). To test whether tuberization was affected by the chlorosis, we examined possible epistatic interactions between the *chlorosis* locus and QTLs detected for tuberization. There was no evidence of such interactions; consequently plants with chlorosis were retained in the BCT data-set.

Results

Relationships among tuberization traits

The analyses of tuberization on apical and two-node cuttings (Ewing 1985) and tuber fresh weight on whole plants are not presented here because QTLs detected for them generally duplicated the QTLs obtained for the three traits that are presented: Cutting-rating (year 1), Cutting-rating (year 2), and % Tuberized plants. An exception was that, as discussed later, an additional QTL was detected on chromosome 4 for tuberization on two-node cuttings.

QTLs identified from the data for the five individual blocks were much the same as those found by taking the mean values for all five blocks. As expected, significance levels for the pooled data tended to be higher. Therefore only the data from the mean values are presented.

The Cutting-rating (year 1) data showed continuous, normal distributions. There was considerably more variability in Cutting-rating (year 1) in BCT than in BCB (Fig. 2), with the standard deviation among genotypes nearly twice as high. Correlations between Cutting-rating (year 1) and Cutting-rating (year 2) were highly significant in both backcrosses (Table 4). In BCT both Cutting-ratings (year 1) and (year 2) were also highly correlated with % Tuberized plants. Correlations among measurements were less marked in BCB, as might be expected in view of the lower variability for tuberization traits among BCB genotypes (Fig. 2). In BCB the correlation data for Cutting-rating (year 1) and % Tuberized plants were based on the entire population, but for Cutting-rating (year 2) there were only 17 selected clones.

Table 4 Correlation coefficients and significance levels for correlations between tested traits. Upper right-half, BCT; lower left-half, BCB. Cutting-rating (year 2) in BCB was based on only 17 selected genotypes; other traits were based upon 155–299 genotypes

	Cutting-rating		% Tuberized plants
	(year 1)	(year 2)	
Cutting-rating (year 1)		+0.67***	+0.58***
Cutting-rating (year 2)	+0.62**		+0.43***
% Tuberized plants	+0.12	+0.09	

** $P \leq 0.01$; *** $P \leq 0.001$

QTLs detected through direct (main) effects

Three QTLs for earliness of tuberization were found in BCT while following alleles segregating from the hybrid parent (Table 5). These were on chromosomes 1, 5, and 6 (Fig. 3). Five other QTLs in BCT were detected while following T^R alleles segregating from the *S. tuberosum* parent used to make BCT (Table 5). These QTLs were located on chromosomes 2, 3, 5, and 8 (Fig. 3). Of the eight QTLs, six were detected by Cutting-rating (year 1) only or in combination with the other traits, whereas 2 QTLs were detected only through % Tuberized Plants (Table 5).

Table 6 shows the cumulative (direct) effects of the 6 loci found in BCT for Cutting-rating (year 1) and the corresponding increases in % Tuberized plants. When unfavorable alleles were present at all 6 loci, the mean rating was only 3.0. If any 3 of the 6 loci had alleles favoring tuberization, then the mean for Cutting-rating (year 1) was 4.5, whereas favorable alleles at all 6 loci produced a mean rating of 7.0. A similar trend was found for % Tuberized plants, which ranged from 13% to 100% with substitution of 0–6 alleles for earliness, respectively. The main difference between the results for Cutting-rating (year 1) and % Tuberized plants was that % Tuberized plants was less discriminating among the genotypes with higher degrees of induction (Table 6). Also, 1 of these 6 QTLs was not significant for % Tuberized plants. The variances explained by the 6 QTLs combined were 53% and 36% for Cutting-rating (year 1) and % Tuberized plants, respectively.

Two QTLs were detected in BCB while following alleles segregating from the hybrid parent (Table 5). They were located on chromosomes 6 and 8 (Fig. 3). No QTLs were found by following B^R alleles segregating from the *S. berthaultii* parent.

Source of alleles associated with early tuberization

Experience in resistance screening and breeding with *S. berthaultii* predicts that the wild species should provide a majority of alleles favoring lateness, or a short-day requirement for tuberization. However, of the 2 QTLs in BCB and the 3 in BCT detected through segregation from the hybrid parent, B alleles promoted earliness in three of the five cases. At marker-locus *TG430b* on chromosome 1, the B allele increased % Tuberized plants by 21% (Table 5). At *TG123* on chromosome 4, homozygous B improved % Tuberized plants by 12% over the heterozygous genotype; and at TG119 on chromosome 6, an increase of 15% for % Tuberized plants was achieved through a B allele (Table 5). Only at *TG413a* on chromosome 5 and *TG261* on chro-

Table 5 Phenotypic effects of the QTLs detected for tuberization traits detected through tests for direct (main) effects. Significance levels are depicted in Fig. 3

a) QTLs detected (LOD>2.5) in BCT through segregation of B alleles from the hybrid parent (TB). The presence or absence of a B allele at each locus is indicated by + or 0, respectively

Chromosome	Locus	Presence of B	Cutting-rating				% Tuberized plants	
			Year 1		Year 2			
			Variance explained (%)	Mean rating	Variance explained (%)	Mean rating	Variance explained (%)	Mean (%)
1	*TG430b*	+	7	5.3	n.s.[a]	–	10	90
		0		4.5		–		69
5	*TG413a*	+	7	4.6	n.s.	–	13	69
		0		5.4		–		92
6	*TG119*	+	n.s.	–	n.s	–	7	87
		0		–		–		72

b) QTLs detected (LOD>2.5) in BCB through segregation of T alleles from the hybrid parent (TB). The presence or absence of a T allele at each locus is indicated by + or 0, respectively

Chromosome	Locus	Presence of T	Cutting-rating				% Tuberized plants	
			Year 1		Year 2			
			Variance explained (%)	Mean rating	Variance explained (%)	Mean rating	Variance explained (%)	Mean (%)
4	*TG123*	+	6	5.0	n.d.[b]	–	14	87
		0		5.3		–		99
8	*TG261*	+	21	5.4	n.d.	–	n.s.	–
		0		4.9		–		–

c) QTLs detected ($P<0.01$) in BCT through segregation of T^R alleles from the recurrent *S. tuberosum* parent (TT). The presence or absence of a T^R allele at each locus is indicated by + or 0, respectively

Chromosome	Locus	Presence of T^R	Cutting-rating				% Tuberized plants	
			Year 1		Year 2			
			Variance explained (%)	Mean rating	Variance explained (%)	Mean rating	Variance explained (%)	Mean (%)
2	*TG234*	+	5	4.6	n.s.	–	6	72
		0		5.3		–		89
3	*TG130*	+	6	4.7	10	4.5	n.s.	–
		0		5.4		5.5		–
3	*TG244*	+	n.s.	–	n.s.	–	7	90
		0		–		–		74
5	*TG441*	+	27	4.0	12	4.3	9	68
		0		5.6		5.4		90
8	*TG41*	+	6	5.4	7	5.5	7	93
		0		4.7		4.7		75

[a] n.s., Not significant
[b] n.d., Not detected

mosome 8 were favorable effects for earliness to tuberize conditioned by alleles originating in *S. tuberosum*.

The direction of allelic effects was similarly unpredictable with regard to segregation from the recurrent *S. tuberosum* parent, which at different QTLs provided alleles for both earliness and lateness. The presence of T^R alleles was associated with later tuberization at *TG234* on chromosome 2, *TG130* on chromosome 3, and *TG441* on chromosome 5, but with earlier tuberization at *TG244* on chromosome 3 and *TG41* on chromosome 8 (Table 5). T^R segregating from the recurrent *S. tuberosum* parent at TG441 decreased Cutting-rating (year 1) by 1.6 units (Table 5).

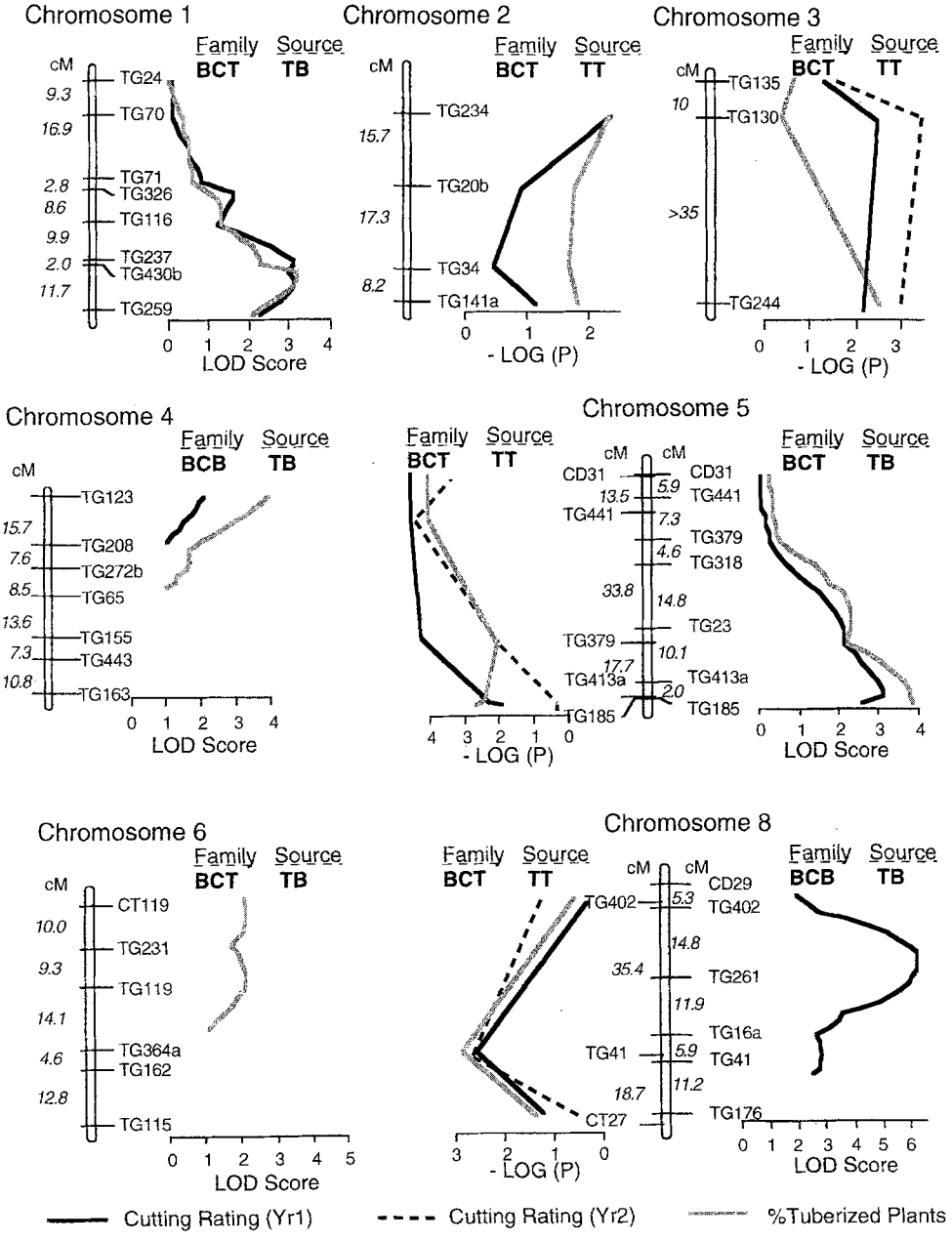

Fig. 3 Chromosome activity maps for tuberization characteristics in BCT and BCB. Maps are based on the segregation of alleles from the hybrid parent in both backcrosses and from the *S. tuberosum* parent used for BCT. The significance levels are shown as LOD scores for alleles segregating from the hybrid, and as -LOG(P) scores (see Materials and methods) for alleles segregating from the recurrent parent of each cross. The datasets to which the activity curves relate are indicated by the backcross family (*BCB* or *BCT*) and parental source of segregating alleles (*TB*, hybrid; *TT*, recurrent *S. tuberosum*). No QTLs were detected with segregating alleles from the recurrent *S. berthaultii* parent (BB)

Table 6 Cumulative effects of alleles favorable to Cutting-rating (year 1) in BCT. The 6 QTLs included (linked to *TG430b*, *TG234*, *TG130*, *TG413a*, *TG441*, and *TG41*) were those for which direct effects were found for this trait. The data for a given number of favorable alleles represent the mean values for all plants that had the given number, regardless of which of the six possible alleles they were

Number of loci with favorable alleles	Cutting-rating (year 1)	Number of observations	% Tuberized plants	Number of observations
0	3.0	2	13	2
1	3.6	19	43	17
2	4.3	26	69	24
3	4.5	41	78	36
4	5.9	34	96	31
5	6.4	14	100	14
6	7.0	6	98	5

The effect at this locus explained 27% of the experimental variance (Table 5).

Epistatic (interactive) effects

All the QTLs that we detected, including those detected for related traits not reported here, were tested for interactive effects with one another. With the large number of tests, it was necessary to guard against chance effects. We are reporting only cases where the interactions for the tested traits had $P \leq 0.01$ for at least one trait, or where the interaction was significant ($P \leq 0.05$) for at least two traits. By these criteria the effects of the QTLs found in BCB were completely additive and explained 21% of the variance for Cutting-rating (year 1) and 14% of the variance

Table 7 Interactions (epistasis) observed in BCT. Interactions presented are those for which at least one trait had $P<0.01$

a) Interactions between two loci both of which had B alleles segregating from the hybrid parent (TB). The presence or absence of a B allele at each locus is indicated by + or 0, respectively

Locus and presence of B	Locus and presence of B	Cutting-rating		% Tuberized plants
		Year 1	Year 2	
TG430b(1)[a]	*TG141a*(2)			
+	+	5.0	4.9	–
+	0	5.7	5.4	–
0	+	4.7	4.8	–
0	0	4.1	4.4	–
(Significance)		(0.005)	(0.09)	n.s.[b]

b) Interactions between one locus that had B alleles segregating from the hybrid parent (TB) with another locus that had T^R alleles segregating from the recurrent *S. tuberosum* parent (TT). The presence or absence of a B allele at the first locus is indicated by + or 0, respectively. The presence or absence of a T^R allele at the second locus is similarly designated

Locus and presence of B	Locus and presence of T^R	Cutting-rating		% Tuberized plants
		Year 1	Year 2	
TG430b(1)	*TG41*(8)			
+	+	5.9	6.0	–
+	0	4.9	4.6	–
0	+	4.5	4.4	–
0	0	4.4	4.7	–
(Significance)		(0.08)	(0.003)	(n.s.)
TRG413a(5)	*TG244*(3)			
+	+	–	–	90%
+	0	–	–	47%
0	+	–	–	91%
0	0	–	–	92%
(Significance)		(n.s.)	(n.s.)	(0.0007)
TG119(6)	*TG135*(3)			
+	+	5.2	5.2	89%
+	0	5.1	5.1	85%
0	+	4.1	3.9	56%
0	0	5.4	5.8	91%
(Significance)		(0.003)	(0.0008)	(0.003)

Table 7 (Continued)

c) Interactions between two loci both of which had T^R alleles segregating from the recurrent *S. tuberosum* parent (TT). The presence or absence of a T^R allele at each locus is indicated by + or 0, respectively

Locus and presence of T^R	Locus and presence of T^R	Cutting-rating		% Tuberized plants
		Year 1	Year 2	
TG135(3)	*TG441*(5)			
+	+	–	3.8	51%
+	0	–	5.3	92%
0	+	–	5.0	85%
0	0	–	5.5	87%
(Significance)		(n.s.)	(0.05)	(0.003)
TG244(3)	*TG65*(4)			
+	+	5.2	–	–
+	0	5.4	–	–
0	+	5.5	–	–
0	0	4.2	–	–
(Significance)		(0.002)	(n.s.)	(n.s.)

[a] (Chromosome number)
[b] n.s., Not significant ($P \leq 0.1$)

for % Tuberized plants. Although in BCT the effects of the QTLs detected also were mainly additive, there were significant epistatic effects. When these interactive loci were included in the regression model, 60% of the variance for Cutting-rating (year 1) was explained, compared to 53% without them. Inclusion of epistatic effects found in BCT for Cutting-rating (year 2) and % Tuberized plants explained 43% and 47% of the variance, respectively, compared to 28% and 38% without them.

Six combinations of markers and traits showed interactions in BCT (Table 7). Of these six combinations, four had in common that only one "favorable" allele, which could be at either locus, was required to cause high values of the given trait. For example, tuberization was favored unless T^R was present at marker *TG135* of chromosome 3 and at the same time B was absent at marker *TG119* of chromosome 6. The opposite of this situation was found in one of the six combinations; i.e., favorable alleles were required at *both* loci to cause a high value. Thus, tuberization was promoted only if a B allele was present at *TG430b* on chromosome 1 *and* TR was present at *TG41* on chromosome 8 (Table 7).

The other interaction was more peculiar. The presence of a T allele at *TG141a* was associated with earlier tuberization when there was a B allele at *TG430b*, whereas it was associated with later tuberization when there was a T allele at *TG430b* (Table 7). An interaction between *TG413a* (chromosome 5) and *CT201b* (chromosome 12 – for map see Bonierbale et al. 1994) showed a similar pattern but is not presented because there was a skewed distribution of progenies with respect to segregation from the recurrent parent. Only 6 genotypes were found that lacked T^R at both *TG413a* and *CT201b*, and only six genotypes had T^R present at both of these markers. In contrast 84 genotypes had T^R present at *TG413a* and absent from *CT201b*, and 54 genotypes had T^R absent at *TG413a* and present at *CT201b*. Skewed distributions in monogenic ratios have been reported by others (Bonierbale et al. 1994; Jacobs et al. 1995) and attributed to the presence of non-viable individuals homozygous for (sub)lethal loci (Jacobs et al. 1995). In the present case the presumed reduction in viability was observed under either the joint presence or the joint absence of T^R at two different loci. The sampling problem associated with this skewing decreases our confidence in the significance of the interaction as a source of variation for tuberization.

Chromosomes with more than one QTL

While common markers were employed wherever possible in both backcrosses, available polymorphism varied; and

this complicates the comparison of results among sources of genetic variability. The QTLs listed in Tables 5 and 7 are associated with 13 markers on seven chromosomes. On chromosome 2 the QTL at *TG234* (Table 5) is probably different from the QTL found at *TG141a*, based on their distant map positions (>30 cM). In contrast, the QTL at *TG135* (Table 7) is only 10 cM from the one at *TG130* (Table 5) on chromosome 3 (Fig. 3), so these two QTLs may be the same. A third QTL on chromosome 3, at *TG244* (Table 5), is well separated from the other two and also presents an opposite phenotypic effect. The presence of T^R was associated with lateness at *TG130*, whereas it was associated with earliness at *TG244* (Table 5). This provides evidence for multiple QTLs affecting earliness on chromosome 3.

On chromosome 4 a QTL close to *TG65* was detected for early tuberization on two-node cuttings (Van den Berg 1993). This QTL, which was highly significant (–log P=3.5), was detected by looking at the effect of T^R alleles in BCT (dataset 2). Further evidence for its role in tuberization was demonstrated by its epistatic effects (Table 7). This QTL most likely was different from the QTL detected for Cutting-rating (year 1) and % Tuberized plants at *TG123* on chromosome 4 (Fig. 3) because the chromosome activity curve for the QTL close to *TG65* was not significant at *TG123* (data not shown), while the activity curve for the QTL close to *TG123* was not significant at marker-locus *TG65*, approximately 32 cM distant (Fig. 3). The 2 important QTLs on chromosome 5 near *TG441* and *TG413a* (Table 5) were separated by more than 30 cM (Fig. 3) and can also be considered to be distinct from one another.

There were also two significant linkage tests on chromosome 8, one in each of the backcrosses. In BCB, B promoted tuberization at *TG261*; and in BCT, T^R promoted it at *TG41*. The question arises as to whether these QTLs tagged the same locus. If so, there must have been at least three alleles at the locus, inasmuch as the genotypes with T alleles from the hybrid were earlier than the genotypes with B alleles for Cutting-rating (year 1) in BCB; but the genotypes with T^R alleles segregating from the *S. tuberosum* parent in BCT were earlier yet (Table 5).

Even if we declare only 1 QTL on chromosome 8, it appears that at least 11 QTLs on seven chromosomes affected tuberization in the two backcrosses.

Discussion

Loci detected for early tuberization

Previous work has established that the cutting response is an excellent predictor of earliness to tuberize, i.e., the ability to tuberize under long photoperiods or high temperatures (Rasco et al. 1980; Ewing 1985; Furumoto et al. 1991). Compared to genotypes with low ratings for tuberization on cuttings, genotypes with relatively high ratings for this trait can be expected to tuberize better, to undergo earlier senescence, and to partition a higher percentage of their dry matter to tubers, especially when the comparisons are made under long photoperiods. Consistent with this conclusion, there was a high correlation in BCT between Cutting-rating (year 1) and % Tuberized plants in the present study (Table 4). Moreover, the QTLs detected by cutting data and by data for percentage of whole plants that developed tubers were generally the same, although loci detected with Cutting-rating (year 1) were often of higher statistical significance. All of the QTLs found for % Tuberized plants were also found for Cutting-rating (year 1), either through direct effects or epistasis. More QTLs were found for Cutting-rating (year 1) than for Cutting-rating (year 2), probably because Cutting-rating (year 1) was an aggregate of more blocks and more cuttings per block than Cutting-rating (year 2). The similarity of QTLs found by Cutting-rating (year 1), Cutting-rating (year 2), and % Tuberized plants strengthens the case that the loci detected are involved in tuberization.

The greater sensitivity of Cutting-rating (year 1) compared to % Tuberized plants was also shown when backcross plants were ranked according to the number of alleles they possessed that favored tuberization. When 6 important loci were included in the ranking, Cutting-rating (year 1) discriminated among all six possibilities, whereas % Tuberized plants could not separate among the presence of four, five, and six favorable alleles (Table 6). This illustrates the importance of having a sensitive test to detect loci of quantitative traits and confirms earlier conclusions (Rasco et al. 1980; Ewing 1985; Furumoto et al. 1991) that cutting ratings are a reliable index of earliness.

Additive and epistatic effects

The aggregate effects of the 6 QTLs identified in BCT for Cutting-rating (year 1) were substantial (Table 6), explaining 53% of the variance. The inclusion of epistatic effects increased the variance explained to 60 % . For many of the epistatic interactions found, one favorable allele was sufficient to benefit earliness; the second allele was not needed. Knowledge of such interactions would simplify the task of breeding for the trait.

Source and dominance of favorable alleles

In both backcrosses we performed linkage analyses for RFLP alleles segregating from the hybrid and the recurrent parents, making it possible to determine whether alleles were segregating from and within both species. Although it is not possible to calculate dominance effects in backcrosses, we were able to make inferences about dominance, based upon whether or not the heterozygous genotype had the favorable phenotype. According to this criterion, early tuberization was dominant, over-dominant (heterotic), or at least partly dominant at 6 of the 11 distinctly different loci affecting Cutting-rating (year 1), Cutting-rating (year 2), or % Tuberized plants.

It is surprising that at 3 of these 11 loci, tuberization was favored by an allele from the wild parent. A similar case of transgressive segregation was observed in an interspecific tomato cross, where favorable alleles segregated from the phenotypically unfavorable wild species *Lycopersicon pennellii*(deVincente and Tanksley 1993).

Of the three *S. berthaultii* alleles that favored tuberization, two were at least partly dominant, since the B-genotypes at these loci tuberized better than the T-genotypes (Table 5). Of the eight *S. tuberosum* alleles that favored tuberization, four – including 1 for two-node cuttings on chromosome 4 (not shown) – were at least partly dominant (Table 5). Early tuberization did not appear to be completely dominant at the loci on chromosome 5, which contained the alleles with the largest effects on tuberization. Heritability studies with other *Solanum* species have led to the conclusion that the most important genes controlling tuberization are recessive (Mendoza and Haynes 1977).

Alleles controlling tuberization were segregating within the *S. tuberosum* species, since 6 of the 10 QTLs in BCT were detected by looking at RFLP alleles that segregated from the *S. tuberosum* parent to which the hybrid was backcrossed. These results illustrate that in an outbreeding species such as potato it is important to look at RFLP alleles segregating from both parents of the cross used for the mapping population (in the present case, the hybrid and the recurrent parents). They also show the importance of differential effects of various genetic backgrounds into which it may be desirable to introgress specific characteristics, both on the trait of interest and on essential horticultural characteristics of the improved germplasm. In this regard, significant progress has already been demonstrated (Furumoto et. al. 1991; Rasco et al. 1980) in selection of early tuberizing germplasm from the short-day-adapted *S. tuberosum* ssp. *andigena*.

Association of trichome-mediated insect resistance and late maturity

There were several coincidences of QTLs for tuberization traits and those reported previously for insect resistance traits. On chromosomes 1, 4, and 6 (Fig. 3) B alleles promoted earliness at *TG430b*, *TG123*, and *TG119*, respectively. Type-A trichome density was favored by B alleles at *TG123* and *TG119* (Bonierbale et al. 1994). Another QTL for resistance to Colorado potato beetle was tightly linked to *TG430b*, and the B allele favored resistance (Bonierbale et al. 1994). These associations help explain why Kalazich and Plaisted (1991) had little problem in combining earliness with type-A trichomes. Not all such associations were favorable, however. On chromosomes 5 and 8 (Fig. 3, Table 5), B alleles conferred lateness and also the desirable condition of increased polyphenol oxidase activity associated with trichome-mediated insect resistance (Bonierbale et al. 1994).

There were also associations between lateness and type-B trichomes. The QTL with the most effect on earliness, detected at *TG441* (Fig. 3), was tightly linked to the most important QTL for type-B trichomes, also detected at *TG441* (Bonierbale et al. 1994). Furthermore, the only QTL detected for the presence on type-B trichomes of visible sucrose-ester droplets was near *TG441* (Bonierbale et al. 1994). The problem for the breeder who is trying to introduce type-B trichomes from *S. berthaultii* is not as impossible as this tight linkage might suggest. The allele that contributed to lateness at *TG441* was not B, but T^R, segregating from the second *S. tuberosum* parent. The presence of a B allele at *TG413a* was associated with lateness, but these two markers are separated by more than 30 cM.

A similar linkage between tuberization and type-B trichome QTLs occurred on chromosome 2 at *TG234* (Fig. 3). Lateness at this QTL was again conferred by a T^R allele; B had no effect (Table 5). Therefore, this linkage should pose no problem in separating type-B trichomes from lateness in our crosses. A weak linkage between earliness and type-B trichomes also occurred on chromosome 1. Earliness was conferred by a B allele at *TG430b* (Table 5), and type-B trichomes were favored by B at a QTL some 50 cM distant (Bonierbale et al. 1994).

The presence on chromosome 5 of the most important allele for type-B trichomes along with two important alleles for lateness helps explain the difficulty of separating lateness from type-B trichomes (Kalazich and Plaisted 1991), especially when one considers that earliness appeared to be recessive at the 2 tuberization loci. In view of the fact that the crop is tetraploid, it is fortunate that six of the other, less important alleles favoring earliness were at least partly dominant. This may explain why it has been possible to select progenies that combine significant levels of insect resistance with maturity earlier than the wild parent and within the maturity range of "main-crop" cultivars (unpublished). Achieving progenies with "first early" maturity would probably require four T alleles at *TG413a*, the absence of lateness alleles at *TG441*, and at least one allele for earliness at each of the loci on other chromosomes. The difficulty of accomplishing this in combination with four B alleles for type-B trichomes at *TG441* underscores the potential value of using RFLP maps to help select parents and screen progenies.

Other implications

Tuber formation is an important survival mechanism for the potato plant. It is therefore not surprising that tuberization is controlled by many different genes, all with relatively small effects. No doubt other genes will be detected when different parents or different environmental conditions are used. The necessity of assembling a large number of genes, many of which are recessive, to obtain earliness helps explain the difficulty of obtaining early maturity in progenies derived from wild species. It is encouraging, however, that, in the crosses investigated, the wild species was able to make its own contribution of alleles for earliness and that some of these are most likely dominant. It is also encouraging that the most common interactive

effects found among genes that controlled maturity meant that the presence of a favorable allele at 1 locus was as effective as favorable alleles at both loci.

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