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Analysis of quantitative trait loci (QTLs) and quantitative trait alleles (QTAs) for potato tuber yield and starch content

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Abstract Using RFLP markers, QTLs for tuber starch-content and tuber yield were mapped in two F_1 populations derived from crossing non-inbred di-haploid potato breeding lines. QTLs were identified and mapped, based on both single-marker tests and interval analyses. A model specifically developed for interval QTL analysis in non-inbred plant species was successfully applied for the first time to experimental data. Results of both methods of QTL analysis were similar but not identical. QTLs for tuber starch-content and tuber yield were analysed in segregating populations K31 and LH in five and two environments, respectively. Population K31 was fully genotyped whereas population LH was selectively genotyped according to high and low tuber-starch content. Eighteen putative QTLs for tuber starch-content were identified on all 12 potato linkage groups and eight putative QTLs for tuber yield were identified on eight linkage groups. Twenty of twenty six putative QTLs were reproducibly detected in at least two environments and/or mapping populations. Few major QTLs for tuber starch-content were highly stable across environments but were detected in only one of the two mapping populations analysed. Most QTLs for tuber yield were linked with QTLs for tuber starch-content suggesting that the effects on both traits are controlled by the same genetic factors. The results are discussed with respect to marker-assisted selection in potato.

Key words Potato · QTL · Tuber starch-content · Tuber yield · Market-assisted selection

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Introduction

The phenotype of most plant characters varies quantitatively as it is under the influence both of the environment and of genetic factors encoded at quantitative trait loci (QTLs, Gelderman 1975). The availability of large numbers of phenotypic neutral DNA markers makes possible the genetic dissection and chromosome assignment of QTLs affecting specific traits. QTL analysis in plants is in most cases carried out in segregating progeny derived from crossing homozygous inbred lines. The cultivated potato is a tetraploid displaying tetrasomic inheritance. Di-haploid lines derived from tetraploids are largely self-incompatible and, therefore, usually not inbred. Several linkage maps of the 12 potato chromosomes have nevertheless been constructed based on segregating progeny of crosses among non-inbred parents using RFLP and AFLP markers (Bonierbale et al. 1988; Gebhardt et al. 1989, 1991, 1994; Jacobs et al. 1995; van Eck et al. 1995). Taking into consideration the non-inbred nature of di-haploid potato, these molecular maps can be used to identify, to map, and to characterize QTLs for agronomic performance. Apart from disease resistance, characters affecting the tuber are considered the most important in potato genetics and breeding. These characters are tuber yield, dry matter, content and quality of starch and protein, cooking and chipping quality, tuber shape, eye depth, flesh and skin colour, taste, glycoalkaloide content, tuberization and tuber dormancy.

Genomic positions for only a few tuber traits have been identified so far using DNA markers. Major loci for tuber flesh-colour and tuber skin-colour have been mapped to potato chromosomes III and X, respectively (Bonierbale et al. 1988; Gebhardt et al. 1989, 1991). Also on chromosome X, a major QTL for tuber shape has been located (Van Eck et al. 1994). QTLs for tuberization, tuber dormancy, chip colour and specific gravity have been analysed in interspecific crosses between di-haploid *Solanum tuberosum* lines and diploid wild potato species (Freyre et al. 1994; Van den Berg et al. 1996a, b; Douches and Freyre 1994; Freyre and Douches 1994). Tuber starch-content and tuber yield are quantitative traits which are easy to determine under field conditions. They are well suited, therefore, to study all aspects of QTL analysis in the potato such as QTL detection in different genetic backgrounds and environments, effects of QTAs (quantitative trait alleles) and methods of QTL detection and location.

We performed a QTL mapping experiment for tuber starch-content and tuber yield using RFLP markers on two different crosses among di-haploid breeding lines. The aims of our study were: (1) to test on experimental data a model developed for interval mapping of QTLs in offspring of non-inbred parents (Schäfer-Pregl et al. 1996), (2) to identify and localize in the potato genome QTLs for tuber starch-content and tuber yield, (3) to analyse the effect of quantitative-trait alleles (QTAs) at specific QTLs, and (4) to assess the stability of QTLs across genetic backgrounds and environments.

Materials and methods

Plant material

Two different populations of F_1 hybrids were analysed. Population K31 was derived from crossing the di-haploid *S. tuberosum* lines H80.577/1 (P3, female parent) and H80.576/16 (P38, male parent). The two parental lines (lines 3 and 38 in Gebhardt et al. 1989) were highly heterozygous as revealed by RFLP markers. Seedling tubers were generated in 1988 and multiplied in 1989. Between 166 and 87 F_1 hybrids – the number depending on year and location – were evaluated for tuber starch-content and tuber yield. Population LH was derived from crossing a diploid interspecific hybrid between *S. tuberosum* and *S. chacoense* (T710) as the female parent with the di-haploid *S. tuberosum* line 45c3 as the male parent. Line 45c3 has been obtained by recurrent selection for high tuber starch-content. In 1990, 451 F_1 hybrids were evaluated for tuber starch-content. Based on this evaluation, 24 high-starch genotypes (H lines, 23–33% starch) and 25 low-starch genotypes (L lines, 11–15% starch) were selected and used for RFLP map construction and QTL analysis.

Phenotypic analysis

In 1990, the K31 population was grown in the field at two locations (K31_S90 and K31_I90): at Scharnhorst (MPI outstation, between 52° and 53° latitude, between 9° and 10° longitude) and at Imola, Italy (between 44° to 45° latitude, between 11° and 12° longitude). Three tubers were planted per plot in three replications. In 1992, the K31 population was propagated again at Scharnhorst (K31_S92) and at Carolinensiel (K31_C92), in an experimental field close to the North sea coast (between 53° and 54° latitude, between 7° and 8° longitude). In 1993, the K31 population was grown at Scharnhorst (K31_S93). At Scharnhorst in 1992 and 1993, 20 tubers were planted per plot in two replications. At Carolinensiel in 1992 five tubers were planted per plot in two replications. At both locations, Scharnhorst and Carolinensiel, tubers were planted between mid April and the beginning of May and harvested during the first week of September (Carolinensiel) and October (Scharnhorst). Two weeks before harvest, plants were treated with the de-foliating agent Reglone.

The LH population was grown at the same location, Imola, in 1990 and 1992 (LH_I90, LH_I92). In 1990, there were three plants per plot and three replications. In 1992, five plants were grown per plot without replication. At Imola, tubers were planted in mid March 1990 and at the end of February 1992 and harvested in mid August 1990 and at the end of July 1992 after the foliage had died. No de-foliating agent was used at the Imola location.

Tuber starch-content in percent was determined by measuring specific gravity (weight in air/weight in air – weight in water) using a starch balance (Meku, Wennigsen, Germany) based on the method of Lunden (1956) for the K31 population; while for the LH population the method of Von Scheele et al. (1937), based on the formula: % starch = 17.546 + 199.07 (specific gravity − 1.0988), was employed. Means for the percent tuber starch-content were calculated over replications. Tuber yield was determined as tuber weight per plot and means were calculated over replications. To obtain normalized values for tuber yield over years and locations, the average tuber yield per plant (g) was calculated.

RFLP analysis and map construction

RFLP markers were selected from approximately 300 marker probes that have been mapped previously (Gebhardt et al. 1994). Selection criteria were genome coverage and polymorphism among the parents of the K31 population (Gebhardt et al. 1989). The majority of marker probes was applied to both the K31 and the LH populations, using the experimental procedures described in Gebhardt et al. (1989). For constructing the K31 map, 157 out of a total of 166 F_1 hybrids were genotyped with the markers. The LH map is based on genotyping 49 selected F_1 hybrids (24 high- and 25 low-starch genotypes). Segregation and linkage of RFLP alleles were analysed as described by Ritter et al. (1990) and Leonards-Schippers et al. (1994). Maps were constructed for the 12 chromosome pairs of both parents of the two crosses using the MAPRF program package developed by E. Ritter.

QTL analysis

Single fragment analysis

Each RFLP fragment segregating in the F_1 populations was scored for presence or absence and for their origin either from parent P_1 or P_2, or from both parents (common fragments). Trait means were calculated for the two subgroups formed according to the presence or absence of the fragment. The means of the two subgroups were tested for significant differences with the two-sample *t*-test. Significance levels were expressed by the probability *P* of no difference between the means compared. Programs were written using SAS software (SAS Institute Inc., 1990) which performed the *t*-test automatically on all fragments scored in the population.

For performing analyses of variance among the phenotypic trait means of four marker-genotype classes at loci where four alleles could be distinguished in the F_1 (two from the P_1 parent and two from the P_2 parent), SAS programs were written using the GLM procedure with the model statement Y = a (SAS Institute Inc., 1989).

The amount of variance explained by allelic differences at a marker locus (R^2) was also calculated.

Interval mapping of QTL

One of the flanking-marker models developed for QTL mapping in non-inbred species, as described in Schäfer-Pregl et al. (1996), was used. The 'four-allele model' considers, at both flanking-marker loci, the segregation in the F_1 of four distinguishable alleles, two from the P_1 parent and two from the P_2 parent, and uses information from the 16 possible F_1 marker genotypic classes, four of which are non-recombinant and 12 are recombinant, to estimate the position of a putative QTL within the marker interval. The model also estimates the trait values of the four F_1 genotypic classes *Q1Q3*, *Q1Q4*, *Q2Q3* and *Q2Q4*, where *Q1*, *Q2* and *Q3*, *Q4* are the quantitative-trait alleles (QTAs) descended from P_1 and P_2, respectively (Leonards-Schippers et al. 1994). Intervals on the 12 linkage groups of the K31 RFLP map were selected that were bordered by RFLP-marker loci (underlined in Fig. 1) which allowed the distinction of four parental alleles. One example of such a highly informative marker locus is shown in Fig. 2. When scored as present or absent (null alleles), two alleles *a* and *b* present in parents P3 and P38, respectively, were sufficient to distinguish the four F_1 marker genotypic classes, *ab*, *a0*, *0b* and *00*. In a few map segments, fully informative marker loci which allowed a distinction between the four F_1 genotypic classes with the same probe were not available (for example, on chromosome V, Fig. 1). In such cases interval borders were based on RFLP loci detected by two different probes but positioned approximately 'opposite' to each other on the two parental maps and known to be linked with a low recombination frequency based on other potato maps. Thus, as an approximation, the RFLP alleles segregating at two such loci were considered as alleles at one interval border locus.

Results

Phenotypic evaluation of tuber starch-content and tuber yield

Population K31 was evaluated for tuber starch-content and tuber yield over 3 years at three locations resulting in five sets of data each for both traits. Population LH was evaluated in 2 years at one location resulting in two sets of data per trait. Trait codes, the number of clones evaluated in each environment (*n*), and the characteristics of the phenotypic distributions obtained in populations K31, LH and in selected subpopulations L and H, are listed in Table 1.

In population K31, the average tuber starch-content (between 10 and 13%) was consistently lower than in population LH (between 19 and 21%). The average tuber yield of population K31 was low at Imola in 1990 (yi1) when compared to the other four environments (yi2, yi3, yi4, yi5). The average tuber yield of subpopulations L and H were significantly different in 1992, with the high-starch lines (H) yielding less than the low-starch lines (L).

Population size decreased from 1990, the first year in the field, to 1992 due to a fraction of genotypes which failed to produce tubers in the field either because plants were of low vigour or because plants were very late maturing and had not tuberized at the time of

Table 1 Environments, trait codes, number of clones analysed per trait (*n*) and statistical parameters for the distributions of tuber starch-content (ts, %) and tuber yield (yi, g/plant) in populations K31, LH and selected subpopulations L and H

Population_environment[a]	Trait code	*n*	Population mean	Standard deviation	Range	Kolmogorov-Smirnov
K31_I90	ts 1	136	10.5	2.6	12.6	> 0.2
	yi 1	136	122.5	78.9	343.5	0.0
K31_S90	ts 2	166	12.2	2.9	14.1	0.05
	yi 2	166	573.8	355.6	1720.8	0.01
K31_S92	ts 3	117	11.0	1.8	10.9	0.20
	yi 3	117	688.9	296.8	1450.0	0.08
K31_C92	ts 4	87	13.2	2.2	11.2	> 0.2
	yi 4	109	946.2	551.3	2860.0	0.01
K31_S93	ts 5	117	10.8	1.4	8.3	0.06
	yi 5	117	570.5	221.4	1042.8	0.01
LH_I90 total	ts	451	19.2	2.8	24.5	0.04
	yi	309	409.7	215.5	1415.6	< 0.01
L_I90	ts 6	25	14.1[c]	0.9	3.4	0.16[b]
	yi 6	25	304.4[d]	232.0	833.3	> 0.2[b]
H_I90	ts 6	24	23.4[c]	2.1	9.7	< 0.01[b]
	yi 6	24	255.5[d]	205.6	909.3	> 0.2[b]
LH_I92 total	ts	317	21.3	2.0	11.0	> 0.2
	yi	306	571.3	302.0	1744.0	< 0.01
L_I92	ts 7	17	19.1[c]	1.5	6.1	> 0.2[b]
	yi 7	17	753.6[c]	312.9	1187.0	> 0.2[b]
H_I92	ts 7	19	23.5[c]	1.5	5.9	> 0.2[b]
	yi 7	18	320.8[c]	160.7	569.0	> 0.2[b]

[a] I = Imola, S = Scharnhorst, C = Carolinensiel, 90 = 1990, 92 = 1992, 93 = 1993
[b] For population size $n \leq 50$, calculation was modified according to Lilliefors (1967)
[c] The difference between means of selected subpopulations L and H was significant at $P \leq 0.05$
[d] The difference between means of selected subpopulations L and H was not significant

harvest. Genotypes not producing tubers were excluded from the analysis. QTL analysis was performed for each data set separately including only those genotypes for which both genotypic and phenotypic data were available (numbers given in the 3rd column of Table 1).

Using the criterion of the Kolmogorov-Smirnov test for normality (Kolmogorov-Smirnov level of significance >0.2), phenotypic variability appeared normally distributed in five of the tuber-starch traits and in four of the yield traits (Table 1). Some distributions could be normalized by transformation. Transformation of the data did not drastically alter the results of the QTL analysis. For comparative reasons therefore, we used untransformed data throughout.

RFLP maps of populations K31 and LH

An overlapping set of 95 and 98 informative RFLP markers was applied to populations K31 and LH, respectively. The numbers scored and the origin of the segregating RFLP alleles are shown in Table 2. An example for segregation in the F_1 generation of two parent-specific alleles and a third allele common to both parents at one RFLP locus is shown in Fig. 2. Four linkage groups were constructed for each of the 12 potato chromosomes, one each for the parental lines of populations K31 and LH using fragments descending from either one or the other parent (Fig. 1). Common fragments were mapped as well as parent-specific fragments. For reasons of clarity, common fragments have not been included in the linkage maps shown in Fig. 1. Many markers were informative for at least one parent in both crosses and could be used, therefore, as anchors for comparing the different linkage maps. Most of the potato genome was covered in both crosses by RFLP loci, with the exception of parts of chromosomes VII and X in population K31 and of chromosomes I, IV and VIII of the 45c3 parent of population LH (Fig. 1). Fewer markers were informative for parent 45c3 indicating a certain degree of homozygosity in this genotype (Table 2).

Table 2 Numbers of the RFLP markers used and the segregating RFLP fragments scored

Item	K31	LH
No. of informative markers	95	98
Fragments descending from the:		
Female parent	103	164
Male parent	103	90
Common	82	66
Total	288	320

Marker order was well preserved in the K31 and LH maps when compared to other potato RFLP maps constructed in different mapping populations (Gebhardt et al. 1991, 1994) with one exception: the order of marker loci *GP167(e)*, *GP171* and *GP92* on linkage group VIII of line T710 was inverted when compared to other maps and no significant linkage was detected to the marker locus *GP40(a)* which in other mapping experiments is linked to *GP92*. This observation may result either from random variation due to the small size of population LH or from a chromosomal rearrangement present in the interspecific parent T710. As observed previously (Gebhardt et al. 1991), recombination frequencies between the same pairs of linked markers in different parental maps were highly variable leading to different lengths of homologous linkage groups (examples are linkage groups III and V). Deviations from the expected segregation ratios were observed on several linkage groups. The self-incompatibility locus on chromosome I, which is closely linked to marker loci *CP100* and *CP108* (Gebhardt et al. 1991), was responsible for the highly distorted segregation ratios observed on linkage group I of the paternal line P38 and the lack of segregation in most of linkage group I of the paternal line 45c3.

QTL analysis based on single fragments

The two-sample *t*-test was performed for all RFLP fragments scored (Table 2) on all traits (Table 1). The percentage of fragments showing a significant effect at $P \leq 0.05$, $P \leq 0.01$ and $P \leq 0.001$ on each of 14 traits (ten and four in populations K31 and LH, respectively) is given in Table 3.

Fragments corresponding to maternal (P3 and T710), paternal (P38 and 45c3) or common RFLP marker alleles that showed – based on the *t*-test – a significant effect at $P \leq 0.01$, $P \leq 0.001$ or $P \leq 0.0001$, were identified on the parental RFLP maps (Fig. 1). The map positions of these fragments are indicated in Fig. 1 by numbers corresponding to the trait codes (Table 1). Effects on tuber starch-content are shown as solid black numbers and effects on tuber yield are shown as open numbers.

Effects on tuber starch-content were detected with RFLP alleles at marker loci on all linkage groups except for group XI of the K31 map and except for groups VIII, IX and XI of the LH map. Effects on tuber yield were detected on all linkage groups of the K31 map except for groups IV, IX and XI and on linkage groups I, III, IV, VII, X and XII of the LH map. Most of the putative QTLs were detected with alleles at several marker loci linked in a particular map segment. QT alleles of both parents contributed to the effects detected in populations K31 and LH (Fig. 1).

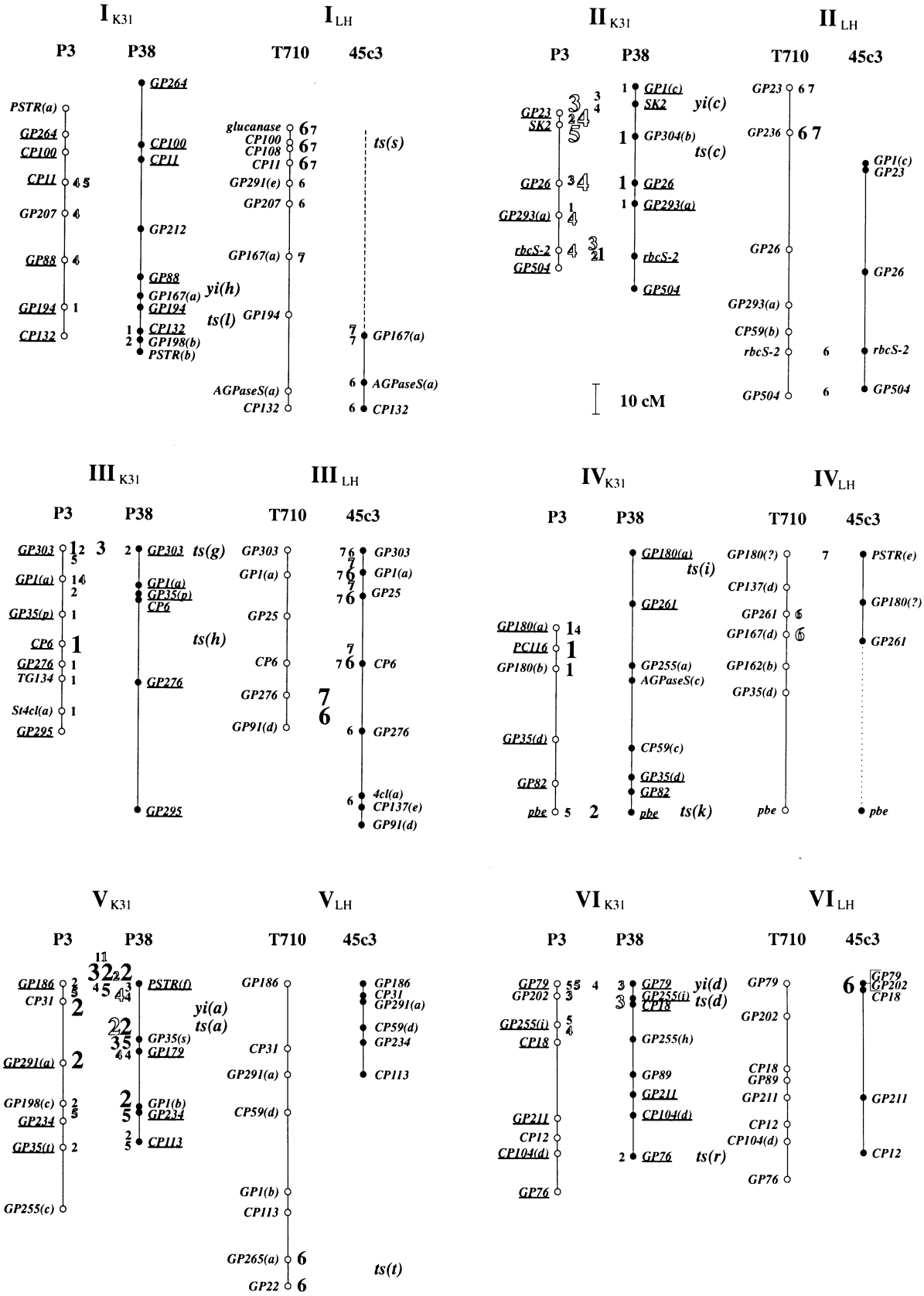

Fig. 1 For legend see page 840

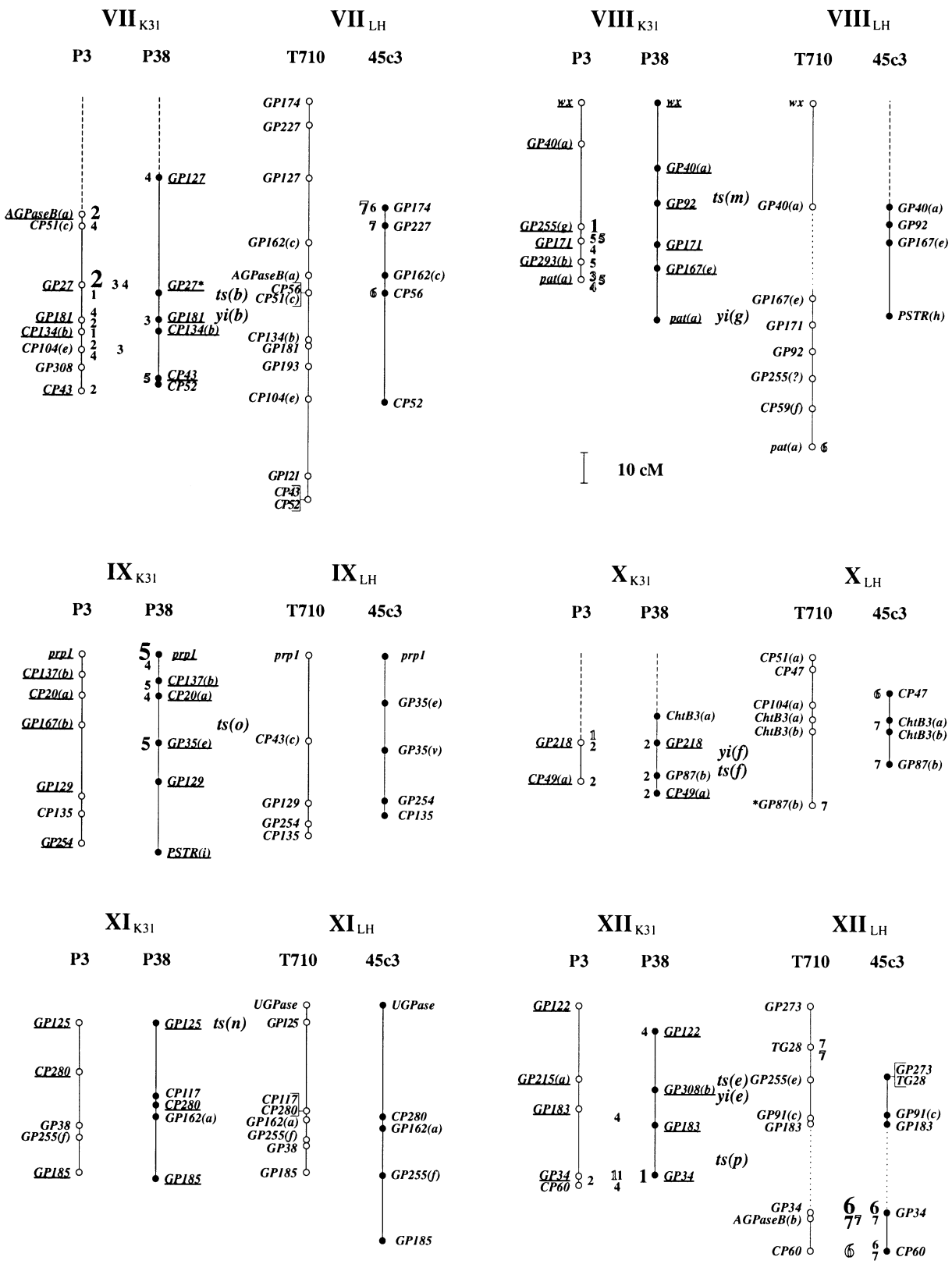

Fig. 1 For legend see page 840

Table 3 Percent RFLP fragments that were significant at $P \leq 0.05$, $P \leq 0.01$ and $P \leq 0.001$ based on the *t*-test performed on all fragments scored (Table 2) using the traits for tuber starch-content (ts1–ts7) and tuber yield (yi1–yi7)

P	K31 population										LH population			
	ts1	yi1	ts2	yi2	ts3	yi3	ts4	yi4	ts5	yi5	ts6	yi6	ts7	yi7
≤ 0.05	26	5	25	10	12	15	21	18	15	12	21	8	21	12
≤ 0.01	14	1	18	5	5	7	11	8	8	5	16	2	13	3
≤ 0.001	5	0	7	1	1	3	1	4	3	2	9	0.3	2	0.3

QTL analysis based on marker intervals of the K31 map

Population K31 was analysed for QTLs using the flanking-marker model considering four alleles at each of the flanking-marker loci (Schäfer-Pregl et al. 1996). Population LH was not considered appropriate for this type of QTL analysis because its size was only 49 individuals, which is too small to occupy the 12 recombinant marker genotypic classes considered by the model with sufficient numbers of individuals. Moreover, trait-based selection for tuber starch-content was applied to this population which is not considered by the four-allele model.

Forty eight marker loci on the K31 map defined 47 intervals – between one and five intervals per chromosome – which are underlined in the K31 linkage groups of Fig. 1. Of 235 intervals tested for the presence of QTLs for tuber starch-content (five traits by 47 intervals) 63 or 26.8% were significant at $P \leq 0.05$. When the same intervals were tested for the presence of QTLs for tuber yield (five traits by 47 intervals), using the same threshold for significance, 31 or 13.2% were significant. Many of the significant intervals were linked and, therefore, not independent. Sixteen putative QTLs for tuber starch-content [*ts*(*a*) to *ts*(*r*)] and eight putative QTLs for tuber yield [*yi*(*a*) to *yi*(*h*)] were assigned to marker intervals of the K31 map (Table 4) based on the following criteria: when adjacent intervals showed an effect, the interval with the maximum and/or over traits the most reproducible effect was assumed to include one putative QTL. In some cases (linkage groups III, IV, VI and XII), when two intervals on the same linkage group showed a maximum effect but were separated by intervals showing no or smaller effects, two putative QTLs were allocated to the same linkage group. Recombination frequencies R1 and R2 between the QTLs and the flanking markers and the trait values for the four QTA combinations *Q1Q3*, *Q1Q4*, *Q2Q3*, *Q2Q4* were also estimated by the four-allele model (Table 4). In cases where the same interval was significant for more then one trait, trait values and recombination frequencies are shown for one trait only (the most significant one or the one where effects on both tuber starch-content and tuber yield were detected). The *ts* and *yi* loci, as defined in Table 4, were included in Fig. 1 at their approximate positions.

QTL analysis by different methods gives similar but not identical results

QTL detection using the *t*-test on single marker alleles at $P \leq 0.01$ and the four-allele model on marker intervals at $P \leq 0.05$ in the K31 population identified largely the same genomic regions harbouring QTLs for tuber starch-content and tuber yield. Exceptions were the QTLs *ts*(*n*) on linkage group XI, *yi*(b) and *yi*(*e*) on linkage group VII and XII, respectively, that were only detected by the four-allele model. By contrast, effects on tuber yield at marker loci *CP11* and *GP207* (linkage group I), and at single marker loci on linkage groups III [*GP1(a)*], VII (*CP43*) and XII (*GP34*), were only significant when using the *t*-test (Table 4, Fig. 1). Some variability between methods was also observed with respect to which of the traits was significant at a particular marker locus or in a particular interval. For example, the QTLs *ts*(*d*) and *yi*(*d*) on linkage group VI were tagged by alleles of markers *GP79*, *GP202* and *GP255(i)* when using the *t*-test on traits ts4 and ts5 for tuber starch-content and on traits *yi3*, *yi4*, *yi5* for tuber yield (Fig. 1). The interval *GP79–GP255(i)* was, however, significant only for traits *ts4* and *yi4* (Table 4).

Fig. 1 Parental RFLP linkage maps of populations K31 and LH including positions of QTLs for tuber starch-content and tuber yield. RFLP alleles from the female (P3, T710) and male (P38, 45c3) parents are shown as *open and closed circles*, respectively; common alleles from both parents are not shown. *Solid black numbers* and *open numbers* next to a marker locus indicate that an RFLP allele at that locus detected an effect on tuber starch and yield, respectively, based on the *t*-test. Traits are coded by numbers *1 to 7* (for trait codes see Table 1 and Materials and methods). *Numbers in the center* between the parental linkage groups indicate that a QTL effect was detected by analysing a common RFLP allele. The significance of an effect is indicated by the size of the numbers: small: $0.001 \leq P < 0.01$; medium: $0.0001 \leq P < 0.001$; large: $P < 0.0001$. Marker loci used to delimit intervals for QTL analysis based on flanking markers are underlined. Putative QTLs for tuber starch-content [*ts(a)–ts(r)*] and tuber yield [*yi(a)–yi(h)*] as defined by interval analysis in population K31 are shown in between the K31 and LH maps at their approximate map positions. Putative QTLs *ts(s)* and *ts(t)* for tuber starch-content on linkage groups I and V, respectively, were found only in population LH

Table 4 QTL analysis in population K31 based on flanking marker intervals and the four-allele model. R1 and R2 are the recombination frequencies estimated between the QTLs in the interval and the flanking markers. Columns 5–8 contain the trait values (ts = % starch, yi = g/plant) estimated for the QTA combinations *Q1Q3*, *Q1Q4*, *Q2Q3*, *Q2Q4*. *P* is the probability that the QTL flanked by the marker loci is detected by chance alone (*: $P \leq 0.05$, **: $P \leq 0.01$, ***: $P \leq 0.001$, ****: $P \leq 0.0001$)

Linkage group	Significant for trait	Interval	R1	R2	*Q1Q3*	*Q1Q4*	*Q2Q3*	*Q2Q4*	*P*	QTL
I	ts 1	*GP194–CP132*	3.0	4.0	10.4	9.3(−)	11.6(+)	10.6	**	*ts(l)*
	yi 4	*GP88–GP194*	4.2	6.2	958	1266 (+)	892	650 (−)	*	*yi(h)*
II	ts 1	*SK2–GP26*	10.6	6.5	9.6	10.3	9.7	12.4(+)	**	*ts(c)*
	yi 2, 3, 4, 5[a]	*SK2–GP26*	0	9.9	1163 (+)	1459 (+)	593 (−)	575 (−)	***	*yi(c)*
III	ts 1, 2, 3, 5	*GP303–GP1(a)*	0	10.2	13.8(+)	12.0	12.0	10.8(−)	***	*ts(g)*
	ts 1	*CP6–GP276*	0	8.2	11.3(+)	11.3(+)	9.8(−)	9.3(−)	**	*ts(h)*
IV	ts 1, 4	*GP180(a)–PC116/GP261*	0	0	14.0(+)	11.8(−)	13.2	14.0(+)	**	*ts(i)*
	ts 2, 5	*GP82–PBE*	7.8	0	10.8	11.4(+)	10.4	10.3	*	*ts(k)*
V	ts 2, 3, 4, 5	*GP186/PSTR(f)–GP291(a)/GP179*	14.9	2.5	8.6(−)	12.5	12.1	14.4(+)	****	*ts(a)*
	yi 1, 2, 3, 4, 5	*GP186/PSTR(f)–GP291(a)/GP179*	13.5	3.9	376 (−)	717 (+)	482 (−)	627 (+)	**	*yi(a)*
VI	ts 4	*GP79–GP255(i)*	11.2	0	14.7(+)	12.2	12.3	13.7	**	*ts(d)*
	yi 4	*GP79–GP255(i)*	0	9.5	1150 (+)	951	996	680 (−)	*	*yi(d)*
	ts 2	*CP104(d)–GP76*	12.8	0	11.9	13.5(+)	11.4	12.7	*	*ts(r)*
VII	ts 1, 2, 3, 4	*GP27– GP181*	9.1	0	10.0(−)	11.3	11.1	11.8(+)	**	*ts(b)*
	yi 3	*GP27–GP181*	9.1	0	687	730	771 (+)	455 (−)	**	*yi(b)*
VIII	ts 1	*GP40(a)–GP255(b)/GP92*	11.1	5.1	11.2(+)	11.8(+)	9.5(−)	10.0(−)	**	*ts(m)*
	yi 3, 5	*GP293(b)/GP167(e)–pat(a)*	0.9	0	330 (−)	615	646	788 (+)	**	*yi(g)*
IX	ts 4, 5	*CP20(a)–GP167(b)/GP35(e)*	8.6	2.9	11.4(+)	9.8(−)	10.7	10.7	***	*ts(o)*
X	ts 2	*GP218–CP49(a)*	7.9	6.1	13.4(+)	12.7	12.6	10.6(−)	***	*ts(f)*
	yi 1	*GP218–CP49(a)*	2.0	10.6	161 (+)	132 (+)	104 (−)	104 (−)	*	*yi(f)*
XI	ts 5	*GP125–CP280*	0	17.5	11.0	11.4(+)	10.9	9.9(−)	**	*ts(n)*
XII	ts 2, 4	*GP122–GP215(a)/GP308(b)*	21.3	0	11.2(−)	13.9(+)	13.6(+)	13.2(+)	**	*ts(e)*
	yi 5	*GP122–GP215(a)/GP308(b)*	21.1	0	530	686 (+)	564	496 (−)	*	*yi(e)*
	ts 1, 2, 4	*GP183–GP34*	10.7	6.7	11.5(−)	13.7	14.4(+)	12.8	**	*ts(p)*

[a] The same interval was significant with all traits indicated; the trait, for which data are shown in columns 4–10, is *underlined*

Linkage between QTLs for tuber starch-content and tuber yield

On the K31 map, QTLs for tuber yield were located in the same intervals or map segments as QTLs for tuber starch-content except for QTLs *yi(h)* on linkage group I and *yi(g)* on linkage group VIII (Table 4, Fig. 1). There were, however, QTLs for tuber starch-content which were not linked to QTLs for tuber yield, mainly on linkage groups III, IV, IX and XI (Table 4). QTL analysis for tuber yield in the LH population was limited by the population size of 49 lines and selection for extremes of tuber starch-content. Effects on tuber yield at $P \leq 0.01$ which were linked to QTLs for tuber starch-content were detected, however, on linkage groups I, III, and VII and at $P \leq 0.001$ on linkage group XII. One putative QTL for tuber yield that was unlinked to tuber starch-content mapped onto linkage group IV (Fig. 1).

Reproducibility of QTLs

Based on anchor RFLP loci mapped in both populations K31 and LH, the positions of QTLs for tuber starch-content were compared across the different genetic backgrounds (Fig. 1). Populations K31 and LH were grown in 1990 in the same mediterranean environment at Imola (traits ts1 and ts6). QTLs were found for these two traits in similar positions on both maps on linkage groups I [*ts(l)*], II [*ts(c)*], III [*ts(g)*, *ts(h)*] and XII [*ts(p)*]. QTLs *ts(g)* and *ts(p)* were also significant in the middle European locations (traits ts2, ts3, ts4, ts5). The QTLs *ts(d)* and *ts(f)* on linkage groups VI and X, respectively, were detected in similar positions on both the K31 and LH maps but in different environments. Some highly significant QTL effects on tuber starch-content were identified in one genetic background only. These were QTLs *ts(a)*, *ts(b)*, *ts(k)* and *ts(o)* on linkage groups V, VII, IV and IX, respectively, of the K31 map and QTLs *ts(s)* and *ts(t)* on linkage groups I and V, respectively, of the LH map (Fig. 1). Whether these QTLs result from differences between genetic backgrounds or from differences between the middle European (Scharnhorst, Carolinensiel) and the mediterranean (Imola) environment is unclear as both populations were tested only once in the same environment. The field experiments have not been designed to study G × E interactions.

Several QTLs for tuber starch-content and tuber yield were detected in more than one environment. The size of effect was variable between environments (Fig. 1). The allele at a particular marker locus by which the effect was detected when using the *t*-test varied also between environments. For example, the three RFLP alleles segregating in population K31 at marker locus *GP303* (Fig. 2, linkage groups III in Fig. 1) detected the QTL *ts(g)* with traits ts1, ts2, ts3 and ts5. The maternal allele *b* was significant with traits ts1, ts2 and ts5, the paternal allele *a* with trait ts2, and the common allele *c* with trait ts3. When using interval analysis, the interval *GP303–GP1(a)* was significant for all four traits (Table 4). In population K31, considering the results of both methods of QTL detection, the QTLs for tuber starch-content detected in three to four of the five environments were: *ts(g)* on linkage group III, *ts(a)* on linkage group V, *ts(b)* on linkage group VII, *ts(m)* on linkage group VIII, and *ts(p)* on linkage group XII (Table 4 and Fig. 1). QTLs for tuber yield which were reproducible over four to five traits were located on linkage groups II [*yi(c)*] and V [*yi(a)*] (Table 4). In population LH, the QTLs for tuber starch-content, stable in both traits ts6 and ts7, mapped to linkage groups I, II, III and XII, corresponding to *ts(s)*, *ts(c)*, *ts(g)–ts(h)* and *ts(p)*, respectively.

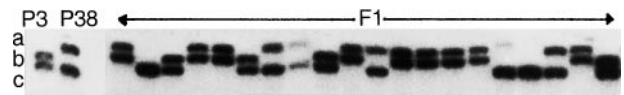

Fig. 2 Example of an RFLP locus (*GP303* on linkage group III) showing the three segregation patterns of marker alleles *a*, *b* and *c* that were scored separately and used for map construction and QTL analysis. The *t*-test was performed for all three fragments separately. In the F_1 offspring, four genotypic classes, *ab*, *a0*, *0b*, *00*, can be distinguished based on scoring alleles *a* and *b* alone as present or absent. Interval analysis of QTLs using the four-allele model was performed with loci where two parent-specific alleles *a* and *b* could be scored

Effect of quantitative trait alleles (QTAs)

Trait values estimated by the flanking-marker model for the four allele combinations *Q1Q3*, *Q1Q4*, *Q2Q3* and *Q2Q4* at the QTLs in population K31 are shown in columns 6–10 of Table 4. Comparing QTA combinations among each other allowed the identification of QTA combinations with favorable, as well as unfavorable effects (indicated by 'plus' or 'minus' in Table 4) on tuber starch-content and tuber yield. Effects were qualitatively, but not quantitatively, consistent relative to each other across traits for *yi(c)*, *ts(g)*, *ts(k)*, *ts(a)* and *yi(a)*, *ts(b)*, *yi(g)* and *ts(o)*, but inconsistent for *ts(i)*, *ts(e)* and *ts(p)* (data not shown). Differences between trait values of QTA combinations of up to 5.8% for tuber starch-content were observed [compare *Q1Q3* and *Q2Q4* at *ts(a)* on linkage group V]. The most favorable QTA combination, *Q2Q4*, for tuber starch-content at this locus was also favorable for yield whereas on chromosome VII, at the linked QTLs *ts(b)* and *yi(b)*, the most favorable QTA combination *Q2Q4* for tuber starch-content had a negative effect on tuber yield. At the major QTLs for tuber yield *yi(c)* on

Table 5 GLM for tuber starch content at selected marker loci of the LH maps. RFLP fragments at the loci selected were significant in the *t*-test (Fig. 1). Analysis was performed for four F_1 genotypic classes distinguished by absence (0) or presence of RFLP fragments (1 denotes presence of P1 = T710-specific RFLP fragment, 2 denotes presence of P2 = 45c3-specific RFLP fragment). At marker loci *CP100* and *GP22* only fragment 1 was scorable. X = phenotypic mean. *P* is the probability that the differences observed are due to chance alone (*: $P \leq 0.05$, **: $P \leq 0.01$, ***: $P \leq 0.001$, ****: $P \leq 0.0001$, ns: not significant). Favorable and unfavorable QTA combinations are indicated by (+) or (−). R^2 is the amount of variance explained at the marker locus

Linkage group	Significant for trait	Marker locus	X_{00}	X_0	X_{02}	X_{10}	X_1	X_{12}	*P*	R^2
I	ts6, 7[a]	*CP100*		16.3(−)			22.4(+)		****	29.7
II	ts6	*GP23*	22.0(+)		18.8(−)	16.2(−)		16.9(−)	**	24.0
III	ts6	*GP303*	15.7(−)		20.1(+)	17.2(−)		20.8(+)	*	19.4
	ts6, 7[a]	*CP6*	19.6(+)		14.3(−)	20.6(+)		16.7(−)	**	22.4
V	ts6, 7[a]	*GP22*		15.9(−)			20.2(+)		**	17.6
VI	ts6	*GP79*	18.4(−)		22.4(+)	18.0(−)		22.2(+)	ns[b]	2.9
VII	ts6	*GP174*	18.8(−)		16.7(−)	21.5(+)		16.3(−)	*	19.5
X	ts7	*GP87(b)*	19.5(−)		21.9(−)	23.2(+)		23.8(+)	***	43.9
XII	ts6, 7[a]	*GP34*	16.8(−)		21.3(+)	16.2(−)		22.2(+)	**	29.6

[a] The marker was significant with both traits indicated. The trait for which X, *P* and R^2 values are shown is *underlined*
[b] The marker genotypic classes X_{02} and X_{12} were occupied by only one genotype each because of highly distorted segregation ratios

linkage group II, trait values (data of trait yi4) for QTA combinations *Q1Q3* or *Q1Q4* were twice the size compared with *Q2Q3* or *Q2Q4*.

In population LH, ANOVA was performed considering the four F_1 genotypic classes at single marker loci linked to QTLs (Table 5). Effects of QTA combinations on tuber starch-content are shown as phenotypic means in Table 5. Exceptions were QTLs linked to *CP100* on linkage group I and to *GP22* on linkage group V where no marker alleles informative for the 45c3 parent (P2) were available. In these two cases only the two maternal genotypic classes were compared. Differences of up to 6% tuber starch-content were observed between means of marker genotypic classes (compare, for example, X_{00} and X_{10} at *GP23* on linkage group II). Favorable and unfavorable QTAs were inherited by both parents resulting in favorable as well as unfavorable combinations of QTAs (indicated by '+' or '−' in Table 5).

Discussion

Methods employed for QTL analysis in non-inbred species

QTLs were detected and mapped using two methods: first, the two alleles at single marker loci of known position on the parental linkage maps were compared and tested for linkage to QTLs by the two-sample *t*-test and, in the case of loci with four alleles distinguishable in the F_1, by GLM (ANOVA). Second, the flanking-marker model considering four alleles per marker locus segregating in the F_1 progeny of non-inbred di-haploid potato parents was used (four-allele model, Schäfer-Pregl et al. 1996). The former method is similar to other QTL studies performed in potato and other outbreeding plant species that are based on single- or flanking-marker models considering two alleles at each marker locus (Douches and Freyre 1994; Freyre and Douches 1994; Freyre et al. 1994; Leonards-Schippers et al. 1994; Van den Berg et al. 1996a, b; Byrne et al. 1997). The latter method takes into account the actual situation present in crosses among non-inbred parents and was applied for the first time to experimental data of F_1 populations derived from crossing heterozygous di-haploid potato lines. The results of both methods of QTL analysis were highly similar although not identical. The best congruence between methods was obtained when comparing results of the *t*-test at $P \leq 0.01$ with results of the four-allele model at $P \leq 0.05$. Few differences were observed concerning the detection of QTLs with small effects and the stability of QTLs across different environments. Using the *t*-test, a QTL may escape detection when the difference between two alleles compared is masked by the other two alleles also segregating, which may also be effective but are not taken into account. The four-allele model may fail to detect a QTL effect when segregation ratios are extremely distorted in one parent as was the case on linkage group I due to the expression of incompatibility at the *S* locus.

The four-allele model allows the positioning of QTLs in a marker interval and the estimation of effects of the four QTA combinations. The model requires, however, a sufficient population size and the availability of sufficient numbers of highly informative marker loci which allow the distinction of the four-marker genotypic classes in the F_1. It also requires prior construction of the linkage map. Forty eight RFLP marker loci covering most of all the linkage groups of the K31 map fullfilled this condition. By contrast, the two-sample *t*-test can be applied to any segregation data based on

scoring the presence versus the absence of a marker fragment. This method of QTL analysis is, therefore, suitable for tagging QTLs in large numbers of segregating fragments as generated, for example, by fingerprinting with AFLP™ (Vos et al. 1995).

QTLs for tuber starch-content and tuber yield

Breeding high-starch potatoes aims at increasing starch yield (kg starch per area unit) rather than increasing tuber starch-content because high tuber starch-content is compromised by a lower yield. This fact was illustrated by the significant difference observed in 1992 between means for tuber yield in selected subpopulations H and L of population LH where lower yield was associated with high tuber starch-content in subpopulation H. QTL analysis based on field evaluations in different environments showed that genetic control of tuber starch-content is complex and involves genes located on all 12 chromosomes. QTLs for tuber yield were smaller in numbers and most of them were found in the same genomic regions that also harboured QTLs for tuber starch-content. Tuber yield was determined by the tuber weight per plant, which is the sum of water content and dry matter. Dry matter-content is tightly correlated with tuber starch-content. Genetic factors which control tuber starch-content may have pleiotropic effects on tuber weight and, therefore, on tuber yield. Such factors can be the common basis of the QTLs for tuber starch-content and tuber yield located in the same genomic regions. Genetic dissection of QTLs affecting only tuber starch-content and QTLs affecting both tuber starch-content and tuber yield, as described in this paper, are the basis for developing marker-assisted strategies to optimize starch yield.

Time to plant-maturity and tuberization are related physiological traits which are controlled by genetic factors and day-length. Potato genotypes which tuberize under short day length conditions in their original habitat, the highland tropics of South America, tuberize late in the season when cultivated under the long-day conditions of middle Europe. Time to plant-maturity and tuberization are also correlated with the accumulation of dry matter or starch in the tubers. We and others (Van den Berg et al. 1996, and unpublished results of our laboratory) in independent mapping populations identified major QTLs for plant maturity and tuberization on linkage group V. Populations K31 and LH have not been evaluated for maturity and tuberization traits. However, the major effects on tuber starch-content and tuber yield found on linkage group V of the K31 map [QTLs *ts(a)* and *yi(a)*] were detected mainly at the locations with a long day-length (Scharnhorst and Carolinensiel, traits 2, 3, 4, 5) where tubers were harvested before late-maturing genotypes may have realized their full yield potential as compared to Imola, where day length was shorter and tubers were fully mature at harvest. These findings suggest that gene(s) with pleiotropic effects on tuberization, plant maturity, tuber starch-content and tuber yield are located on potato chromosome V. Also in population LH, which was evaluated at Imola only, QTLs *ts(a)* and *yi(a)* were not detected. This may be either due to homozygosity at the corresponding loci in the LH population or due to the difference in day length and harvest conditions between the locations.

Stability of QTLs

In the germplasm analysed, none of the QTLs were detected in all environments and in both populations K31 and LH. However, with the exception of six of 26 putative QTLs [*ts(t)*, *ts(r)*, *yi(b)*, *yi(f)*, *ts(n)* and *yi(e)* on linkage groups V, VI, VII, X, XI and XII, respectively] the QTL effects were reproducible at least once either in both populations K31 and LH, in different environments within the same population, or in both.

A QTL mapping experiment for specific gravity in tubers has been carried out on an interspecific cross between diploid potatoes (Freyre and Douches 1994). As tuber starch-content is determined by measuring specific gravity, both Freyre and Douches (1994) and ourselves studied essentially the same trait in different genetic materials. In the experiment of Freyre and Douches (1994), QTLs for specific gravity were identified on chromosomes I, II, III, V, VII and XI using isoenzyme, RAPD, and tomato as well as potato RFLP markers (Gebhardt et al. 1991; Tanksley et al. 1992). Although the markers used in the study of Freyre and Douches (1994) and in our experiments were different, some isoenzyme and RFLP markers make an indirect comparison of QTL map positions possible based on the collinearity between the potato and tomato linkage maps (Bonierbale et al. 1988) and the alignment of our potato RFLP map (Gebhardt et al. 1991) with the tomato/potato RFLP maps of Tanksley et al. (1992). This comparison suggests that both research groups independently and in different genetic materials detected similar QTLs for specific gravity/tuber starch-content on linkage groups I and III, possibly on linkage groups II and particularly the QTLs on linkage groups V and VII. In another study (Bonierbale et al. 1993), three tetraploid potato populations were analysed with tomato RFLP markers of known position on the molecular map of tomato. Effects on specific gravity and tuber yield were detected with markers positioned on tomato chromosomes 1, 2, 4, 5, 7, 9 and 12. Although a comparison of QTL positions is more difficult in this case, because of the lack of suitable reference markers, some of the QTLs detected by Bonierbale et al. (1993) may again be the same as in the QTL mapping experiments carried out in diploid potatoes. Our results and those of others (Bonierbale et al. 1993; Freyre and Douches 1994) support the notion that genetic factors

reproducibly affecting the starch-content of tubers are located mainly on chromosomes I, II, III, V and VII of potato.

Marker-assisted selection for tuber starch-content and tuber yield

Based on two F_1 populations, we have characterized QTAs for tuber starch-content and tuber yield present in four diploid potato breeding lines. Freyre and Douches (1994) and Bonierbale et al. (1993) performed QTL analysis for specific gravity/tuber starch-content in other populations derived from crossing diploid and tetraploid clones not related to the parents we employed. Within the germplasms analysed so far, marker-assisted strategies can be developed to pyramidize the QTAs present in these materials taking into consideration genomic position, size and reproducibility of the effect, and epistatic interactions among QTAs at selected loci. For genotyping large numbers of plants with a reasonable investment of time and resources, RFLP markers tightly linked to QTLs can be converted into SCAR markers which are analysed by PCR (Ballvora et al. 1995; Meksem et al. 1995; Niewöhner et al. 1995). The availability of microsatellite markers of known genomic position (Milbourne et al. 1998) will provide another source of locus-specific, PCR-based markers. Selection strategies and marker alleles to be selected for or against will be different, however, depending on which combinations of genotypes are used. The predictive value of the QTL analyses performed so far is restricted to the positions of QTLs in the potato genome. In unrelated germplasm, including tetraploid germplasm, the positions of several major QTLs affecting tuber starch and yield are likely to be the same as in the materials analysed to-date (see above). However, which QTAs are present at those loci cannot be predicted and their effect will have to be evaluated before marker-assisted selection can be applied. This evaluation should become easier, more efficient, and amenable to automatization when new marker technologies such as AFLP™ (Vos et al. 1995) are employed. QTAs in tetraploid potatoes may be tagged when large numbers of AFLP fragments are scored for presence or absence and tested for effect by a single-marker test like the *t*-test. AFLP fragments having a significant effect on the trait of interest can be physically isolated and mapped as conventional RFLPs on existing diploid mapping populations (Meksem et al. 1995), in this way determining the position of the QTLs showing the effect detected by the original AFLP marker. AFLP fragments of identical size which are present in unrelated germplasm have a good probability of identifying the same locus (Van Eck et al. 1995; Rouppe van der Voort et al. 1997). In cases where such AFLP fragments also show a similar effect on the trait of interest, it may be concluded that the AFLP fragment is in fact, tightly linked to the same QTA.

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