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Localization of QTLs for tolerance to *Cercospora beticola* on sugar beet linkage groups

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Abstract We present a new linkage map for sugar beet (*Beta* vulgaris) which has been developed using a population segregating for genetic factors that confer tolerance to the leaf spot fungus (*Cercospora beticola*), the causal factor of leaf spot disease in sugar beet). In the F_2 population studied, a subset of 36 RFLP probes, mapping on eight out of the nine linkage groups of sugar beet, provided the anchor markers to assign chromosomes. A total of 224 markers, including RFLPs, AFLPs, SCARs and microsatellites, were mapped. Estimates of leaf damage in F_2 and test-cross families were repeated at different stages of plant development. Each set of data was analysed as such. An average estimate was also considered. QTLs with highly significant LOD scores revealed both by the F_2 and test-cross analyses were localized on linkage groups 2, 6 and 9. Linkage groups 4 and 5 gave a clear indication of the presence of a QTL only when F_2 data were considered. One highly significant QTL with a LOD of 16.0 was revealed only by the data obtained under conditions of artificial inoculation. This QTL maps at position 90 on chromosome 3.

Keywords Resistance · Sugar beet · Leaf spot disease (*Cercospora beticola*) · Genetic linkage map · QTLs

Introduction

Leaf spot, caused by the fungus *Cercospora beticola*, is an important sugar beet (*B. vulgaris*) disease in western Europe. The phytotoxic metabolite *C. beticola* toxin (CBT, Schlösser 1962, 1971) induces a state of necrosis which may extend to 60% of the leaf surface. Loss of leaf area accelerates the growth of new leaves and causes redirection of assimilation products and up to 50% loss of sugar yield (Geisler 1980, Wolf et al. 1995). Due to the current reduction in soil tillage and shorter cycles of crop rotation, residues of sugar beets are becoming an efficient source of inoculum for leaf spot disease (Schäufele and Wevers 1996, Hartleb et al. 1997). Although efficient pest management can control the disease, the intensive use of fungicides stimulates the emergence of resistant strains of the fungus (Kato et al. 1984; Hermann and Meeus 1996).

The use of resistant cultivars of sugar beet is an attractive alternative because it reduces both production costs and the impact of pesticides on the environment. However, the breeding of resistant cultivars is difficult owing to the polygenic inheritance of this trait (Mesbah et al. 1997). The advent of DNA markers has made it possible to map discrete genetic factors associated with a quantitatively inherited trait of interest. Such loci are called quantitative trait loci (QTLs, Geldermann 1975). Linkage maps based on restriction fragment length polymorphism (RFLP) and amplified fragment length polymorphism (AFLP) markers of diploid, segregating populations have already been published for sugar beet (Barzen et al. 1992, 1995; Pillen et al. 1992; Schondelmaier et al. 1996; Vos et al. 1995). In this paper, we present a new linkage map for sugar beet which was developed from a population segregating for genetic factors that confer tolerance to the leaf spot fungus. The aim of the study was (1) to test the applicability of the AFLP markers for constructing a linkage map in sugar beet, (2) to identify QTLs affecting resistance to leaf spot disease and (3) to examine the stability of mapped QTLs to expression of resistance in different environments.

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Materials and Methods

Plant material

An F_2 population – K2 (P952) – consisting of 211 single plants was developed by Kleinwanzlebener Saatzucht AG (KWS), Ein-

beck, Germany. The population was derived from a cross between a plant of an inbred line (KWS no. 90777) that is susceptible to leaf spot disease and a plant from a resistant line (KWS no. 90756). Among the F_1 progeny, one plant was selected, cloned and selfed to assemble the F_2 progeny. A total population of 211 F_2 plants was obtained; 185 progenies were tested as F_3 families for resistance to leaf spot disease. In parallel a mixture of F2 clones and F3 families were crossed to a common tester, thus generating a test-cross population (TC) of 181 progenies.

Phenotypic data

Field data were assembled by KWS at Monselice (Italy) and at Einbeck and Seligenstadt (Germany) in 1996 using a field arrangement of a 10×10 Latin-square design with four and three replications, respectively, for the F_2 and TC populations. Estimates of leaf necrosis were based on a 9-point scale following the standard method used at KWS (KWS 1963). In Italy, the F_2 and test-cross populations were evaluated under conditions of natural *Cercospora* infection.

Monselice is located in the eastern Po Valley where sugar beet is regularly exposed to epidemic attacks of *Cercospora*. In this area, the disease prevalence (percentage of total area of sugar beet infected) every year is constantly much higher than 90% (Rossi et al. 1995). In the same area, the disease index (which integrates classes of disease intensity in different parts of the region to obtain a value expressed a percentage of the maximum possible) is the highest of Europe, with values always higher than 60% (Rossi et al. 1995); 100% corresponds to 100% of the region cultivated with sugar beets having maximum symptoms, i.e. leaves totally dried. Canova et al. (1994) reported, for example, that in a six-location experiment carried out in the Po Valley in 1991, 1992 and 1993 the symptom values of 27 varieties genetically different in resistance to *Cercospora* had coeffients of correlation equal to 0.92 (1991 vs. 1992), 0.86 (1991 vs. 1993) and 0.89 (1992 vs. 1993). It is thus evident that in the Po Valley sugar beet varieties can be scored in 1 year for *Cercospora* susceptibility, with a large margin of security in genotype evaluation.

Estimates of *Cercospora* infection of the F_3 families were made weekly at five dates during cultivation, starting at the beginning of August (*CF2-1* to *CF2-5*). Mean values were also calculated across estimates and treated as a single trait in QTL analysis (*CF2-M)*. For the TC population the level of damage was evaluated every week from the beginning of August (*CTC-1* to *CTC-4*), and mean values were also derived (*CTC-M*).

In Einbeck, the TC progenies were artificially inoculated using a pathogen source cultivated in vitro, homogenized and applied as an aqueous suspension according to Adams et al. (1995). A first inoculation on the 12th of July was followed by a second one on the 6th of August. The degree of leaf necrosis was estimated once (*CTC-G*).

RFLP and AFLP analysis

A total of 260 RFLP probes mapped in previous studies (Barzen et al. 1992, 1995; Pillen et al. 1992; Schondelmaier et al. 1996) were screened at Planta GmbH, Einbeck in order to detect polymorphisms in the *K2* population. All in all 80 probes were polymorphic and chosen for mapping.

For AFLP analysis, one HIND/ATA and 16 MSE primers (MSE31–MSE46) were combined.

Scoring and map construction

The symbols *A* and *B* were assigned to RFLP bands when segregating F_2 plants were, respectively, homozygous for marker alleles inherited from parent 1 or from parent 2. *H* was assigned to plants heterozygous for alleles of polymorphic loci derived from both parents. Bands not identified unambiguously were regarded as missing values.

In the population, AFLP bands were scored as dominant. The absence of an amplified fragment detected in parent 1 (two null AFLP alleles) was indicated by *A.* Absence of an amplified fragment detected in parent 2 was indicated by *B. C* corresponds to the presence in F_2 plants of the fragment inherited from parent 2, in either the homozygous or heterozygous form. *D* was assigned to cases in which the fragment derived from parent 1 was present in either the homozygous or heterozygous configuration.

Linkage analysis and map construction was performed using the software package MAPMAKER 3.0 (Lander et al. 1987). For a few polymorphisms, linkage phase could not be identified due to incomplete homozygosity of the parental strains. To assess linkage of these markers, which were partially in repulsion, we inverted the complete set of data, assigning marker alleles to F_2 plants on the basis of the opposite assumption of parental origin; e.g. *A* instead of *B*, and *C* instead of *D* (M. Daly, MIT, Mass., personal communication). The original and inverted data sets were processed simultaneously in one MAPMAKER-compatible raw data file. Marker symbols in the inverted data set start with an additional 'r'. RFLP markers already placed on linkage groups in the mapping experiments of Barzen et al. (1992, 1995) and Pillen et al. (1992) were used as anchor markers to identify linkage groups. Assignment of chromosomal numbers to linkage groups was according to Butterfaß (1964), Schondelmaier and Jung (1997). The Kosambi (1944) mapping function was used. Linkage of other markers to linkage groups defined by anchor markers were detected using the 'assign' command of MAPMAKER with repetitions at LOD levels 5.0, 4.0 and 3.0.

When two corresponding linkage groups, assembled from the original and inverted data sets, gave identical results, this was taken as proof of correct marker assignment. The set of linkage groups generated by the inverse assignment of marker alleles was discarded from the analysis after completion of grouping. For eight out of nine chromosomes, the 'order' routine of MAPMAKER was sufficient to order markers along the linkage groups based on a starting order with a log-likelihood ratio of 3.0. For one group, the order was based on the 'compare' procedure of MAPMAPER, followed by the 'build' command. A unique placement of markers was performed starting with a LOD threshold of 5.0 and ending at a LOD level of 2.0. Markers which could not be ordered uniquely were placed relative to the map. Linkage groups were drawn using the 'draw chromosome' function of MAPMAKER.

QTL analysis

The analysis of QTLs for leaf spot resistance was based on the software package QTL CARTOGRAPHER V1.12F (Basten et al. 1994, 1997). In order to enhance the accuracy of the localisation of QTLs, the composite interval mapping approach of Zeng (1993, 1994), which combined interval mapping (Lander and Botstein 1989) with multiple regression analysis (Cowen 1989; Stam 1991), was used. The MAPMAKER – 'maps' and 'raw' files containing the map information and the phenotypic data, were translated by the QTL CARTOGRAPHER programes 'rmap' and 'rcross', respectively, to CARTOGRAPHER format. With the programes 'srmap.qtl' a forward regression with backward elimination (FB) method was performed, in which only markers with a significance of $P<0.1$ for the trait of interest were considered. The markers were sorted by their significance and stored by the programme to be used as background parameters in the composite interval mapping programme 'zmapqtl'. Within this programme, the number of background parameters to be considered was set to ten, and the walking speed to 2 cM. The window size, representing the region around the test site not considered as background, was set to 5 cM. The programs 'eqtl' and 'preplot' were used to prepare the graphical output of QTL LOD curves, which were produced by the plotting package GNUPLOT. The threshold LOD score of 2.5 was chosen for a locus to be considered a QTL.

Results

Linkage map

In the F_2 population studied, 260 RFLP probes mapped in previous investigations were tested. Of these, 80

Table 1 Markers scored and placed on sugar beet linkage groups

Marker	Number scored	Number mapped
RFLP	80	75
AFLP	180	146
SCAR	3	2
Microsatellite	1	1
Total	264	224

Table 2 Distribution of RFLP and AFLP markers assigned to the nine linkage groups of sugar beet

Linkage groups	Number of markers assigned[a]			
	RFLP		AFLP	
	a	b	a	b
1	1	0	8	3
2	7	0	17	9
3	6	0	9	4
4	10	1	9	6
5	11	0	7	4
6	8	0	11	15
7	8	1	11	6
8	7	1	7	8
9	12	2	9	5
Total		5		

[a] a, Assigned uniquely to one position at LOD 2; b, placed at the most probable of the possible positions on a particular linkage group

showed polymorphisms. A subset of 36 probes mapping on eight of the nine linkage groups of sugar beet provided the anchor markers. A few of the probes mapped in earlier studies generated conflicts when assigned to the same linkage groups in this investigation. These could be assigned with higher LOD scores to other linkage groups. Five RFLP markers showed no linkage to any group. None of the RFLP anchor probes were assigned to linkage group 1. Linkage group 1 only became evident when the *group* command of MAPMAKER assembled almost all unassigned markers together as one group in addition to the eight groups already successfully identified.

Of a total of 264 markers scored, including RFLPs, AFLPs, SCARs (sequence characterized amplified region) and microsatellites, 224 were mapped (Table 1). Out of 75 RFLP markers assigned to linkage groups 72 could be placed at one defined position on the linkage map; five had more than one possible position and were placed at the one with the highest probability (Table 2). One additional microsatellite marker was mapped to a distal position on linkage group 7. Three SCAR markers were placed on linkage group 3 (Fig. 1).

The AFLP analyses was carried out with 16 primer combinations on 211 F_2 genotypes: 180 polymorphic fragments were scored, and 176 AFLP markers could be assigned to linkage groups. Ordering of these 176 AFLP markers along the linkage groups assigned 88 markers to specific positions (a in Table 2); a further 60 AFLP markers were mapped at the most probable of the possible positions on a particular linkage group (b in Table 2), 30 markers could not be mapped.

Table. 3 Means and their variances of the 12 estimates of *Cercospora* resistance as evaluated under natural infection conditions in Italy and with artificial inoculation in Germany. Resistance scores were assigned according to a 9-point scale, with 1=no infection and 9=100% leaf area infected (*CF2* F_2 population. *CTC* test-cross population)

Trait	Mean	Variance
CF2-1	3.11	1.06
CF2-2	5.05	0.92
CF2-3	6.53	0.95
CF2-4	8.31	0.39
CF2-5	8.68	0.14
CF2-M	6.34	0.53
CTC-1	3.41	0.31
CTC-2	4.02	0.19
CTC-3	5.83	0.14
CTC-4	8.07	0.62
CTC-M	5.35	0.16
CTC-G	5.04	0.77

The ordering of markers on linkage groups started in almost all cases with RFLP markers, except for linkage group 1, where the starting order was based on only 1 RFLP and 3 AFLP markers. Ordering in this case was performed using the MAPMAKER command 'compare'. Some clustering of both RFLP and AFLP markers was observed for most of the linkage groups, particularly for groups five to seven and nine.

QTL analysis of *Cercospora* tolerance

Twelve sets of *Cercospora* data were available: CF2-1 to -5 + M for F_2 plants in Italy, CTC-1 to -4 + M for TC progenies in Italy and CTC-G for the TC population in Germany. Means and variances for the 12 traits are reported in Table 3. Means for *CF2* increased from 3.11 for the first estimate to 8.68 for the last estimate. CTC values ranged from 3.41 to 8.07 (CTC-4). As expected, variances of the *CF2* series were, on average, higher than those of the *CTC* series.

Table 4 reports the results of the QTL analyses. Before considering these results we first should review some concepts. Leaf damage estimates were repeated at different stages of plant development. Each set of data (CF2-1 to -5 and CTC-1 to -4, CTC-G) was analysed as such. Also, average estimates were used (CF2-M, CTC-M). The finding of a high LOD value at a position on a particular linkage group based on average *Cercospora* resistance supports the existence of a QTL. This finding has more weight when (1) for the same position LOD values are also highly significant for average scores from the TC population, and (2) when for both the F_2 and TC populations LOD scores based on plant-stage-specific estimates are also significant for the presence of a QTL at the same position. A QTL revealed by the analyses carried out on F_2 data, however, may not be confirmed in the TC population. In cases where the LOD scores of a

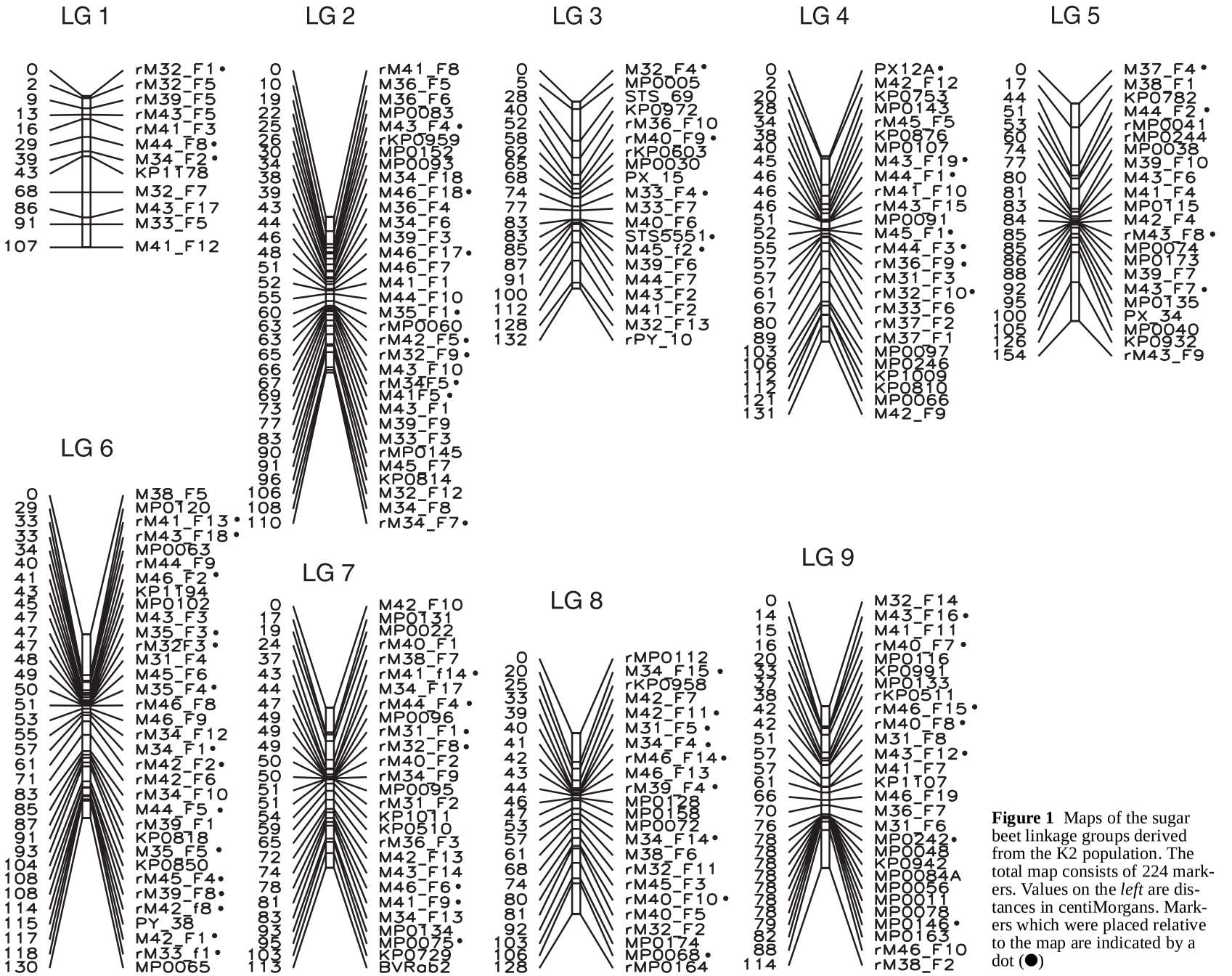

Figure 1 Maps of the sugar beet linkage groups derived from the K2 population. The total map consists of 224 markers. Values on the *left* are distances in centiMorgans. Markers which were placed relative to the map are indicated by a dot (●)

Table 4 Location and LOD scores of QTLs for leaf spot disease mapping on nine sugar beet linkage groups for F_2 and test cross progenies tested in different locations or at different times during the growth period

Trait	Chrom 1		Chrom 2a		Chrom 2b		Chrom 3		Chrom 4		Chrom 5		Chrom 6		Chrom 7		Chrom 8		Chrom 9	
	cM[a]	LOD[b]	cM	LOD	cM	LOD	cM	LOD	cM	LOD	cM	LOD	cM	LOD	cM	LOD	cM	LOD	cM	LOD
CF2-1	–	n.s.[c]	30–50	**4.5**	85–110	**4.5**	105–125	**3.0**	30–55	**6.0**	-	n.s.	60–80	**11.0**	–	n.s.	50–80	**3.0**	–	n.s.
CF2-2	–	n.s.	30–50	**6.0**	65–115	**8.5**	–	n.s.	30–55	**3.0**	60–75	**3.5**	70–105	**6.5**	–	n.s.	0–10	**3.5**	70–85	**3.5**
CF2-3	–	n.s.	30–55	**5.5**	70–115	**9.5**	–	n.s.	30–55	**3.0**	60–75	**3.5**	60–105	**7.5**	–	n.s.	0–20	**3.5**	65–90	**5.0**
CF2-4	–	n.s.	–	n.s.	85–115	**15.0**	–	n.s.	0–20	**7.0**	50–75	**4.5**	70–105	**4.0**	70–90	**3.0**	–	n.s.	65–90	**9.0**
CF2-5	50–110	**4.0**	–	n.s.	85–115	**10.0**	–	n.s.	0–20	**7.5**	60 – 125	**5.0**	0–40	**7.0**	60–90	**4.5**	–	n.s.	65–80	**2.5**
CF2-M	–	n.s.	30–55	**6.0**	80–115	**8.0**	–	n.s.	30–55	**4.0**	60–75	**3.0**	60–80	**8.5**	–	n.s.	0–10	**3.5**	65–85	**4.5**
CTC-1	–	n.s.	0–25	**4.5**			90	**2.5**	–	n.s.	75	**2.5**	45–65	**2.5**	–	n.s.	–	n.s.	70 – 80	**2.5**
CTC-2	–	n.s.	10–20	**3.5**			–	n.s.	–	n.s.	75	**2.5**	–	n.s.	–	n.s.	–	n.s.	80–120	**4.5**
CTC-3	–	n.s.			95–115	**3.5**	–	n.s.	–	n.s.	–	n.s.	–	n.s.	60–90	**4.5**	–	n.s.	70–80	**3.5**
CTC-4	–	n.s.	–	n.s.	–	n.s.	–	n.s.	–	n.s.	45–60	**3.5**	0–45	**5.5**	60–90	**4.0**	30–55	**4.0**	–	n.s.
CTC-M	–	n.s.	0–20	**3.5**	–		–	n.s.	–	n.s.	–	n.s.	35–55	**4.5**	0.75	**4.5**	15–30	**3.0**	60–80	**5.5**
CTC-G	–	n.s.	25–50	**4.5**			70–115	**16.0**	–	n.s.	–	n.s.	30–65	**10.5**	-	n.s.	–	n.s.	60–120	**5.0**

[a] Distance of the QTL from the left telomere in centiMorgans. The distances were taken as corresponding to the intercept of the QTL LOD score line with the line representing the LOD 2.5 threshold of significance

[b] Significance level expressed as logarithm of likelihood ratio; significant values are displayed in bold

[c] Levels below LOD 2.5 were considered not significant (n.s.)

putative QTL are significant only for F_2 data, one possible interpretation is that the QTL allele segregating in the F_2 population is recessive.

QTLs with highly significant LOD scores in both the F_2 and test cross analyses were localized on linkage groups 2, 6 and 9. A significant QTL was found on linkage group 2, 10–40 cM from the left telomer (2a in Table 4). The presence of this QTL was evident from both F_2 and TC data, regardless of whether resistance was tested under natural or artificial inoculation conditions. The LOD scores for this QTL peaked at positions around 40 cM in the F_2 data and around 10 cM in the TC data. On the same linkage group a second QTL with very significant LOD scores was found some 100 cM from the left telomer, (2b in Table 4). However, the available TC data did not confirm the presence of the QTL at this position (with the exception of the data from estimate 3). A likely interpretation is that this QTL allele associated with resistance to leaf spot disease is not segregating in the TC population.

A significant QTL was also mapped at region 40–80 on linkage group 6, based on F_2 and TC estimates and on natural and artificial inoculations. However, the TC data supporting for this QTL were less robust, particularly when natural inoculation was considered. Nevertheless, for this QTL, LOD values based on average estimates were always highly significant (8.5, 4.5 and 10.5, respectively, for CF2-M, CTC-M and CTC-G).

The F_2 and TC data for linkage group 9, collected under both natural and artificial infestation conditions, indicated with high probability that a QTL is present near position 75.

Linkage groups 4 and 5 each gave clear indications of the presence of a QTL only when F_2 data were considered. These two QTLs were, respectively, located at positions 40 and 70. The QTL on linkage group 5, nevertheless, is also discernible from TC data (estimates CTC-1, 2 and 4).

One highly significant QTL with a LOD of 16.0 was revealed only by the data collected in Germany under conditions of artificial inoculation. This QTL maps on chromosome 3 at position 90.

Figure 2 shows a graphical representation of the LOD value distribution which assigns major QTLs to chromosomes 2. It shows also positions where the QTL LOD score line intercepts the LOD 2.5 threshold line: the intercepts were used to calculate the QTL intervals reported in Table 4.

Discussion

Linkage map

Mapped RFLP markers are an obvious choice for assignment of linkage groups when constructing a new map for a crop species. Once a balanced set of anchor probes has been defined, MAPMAKER allows one to assign new markers to each linkage group. The advantage of this

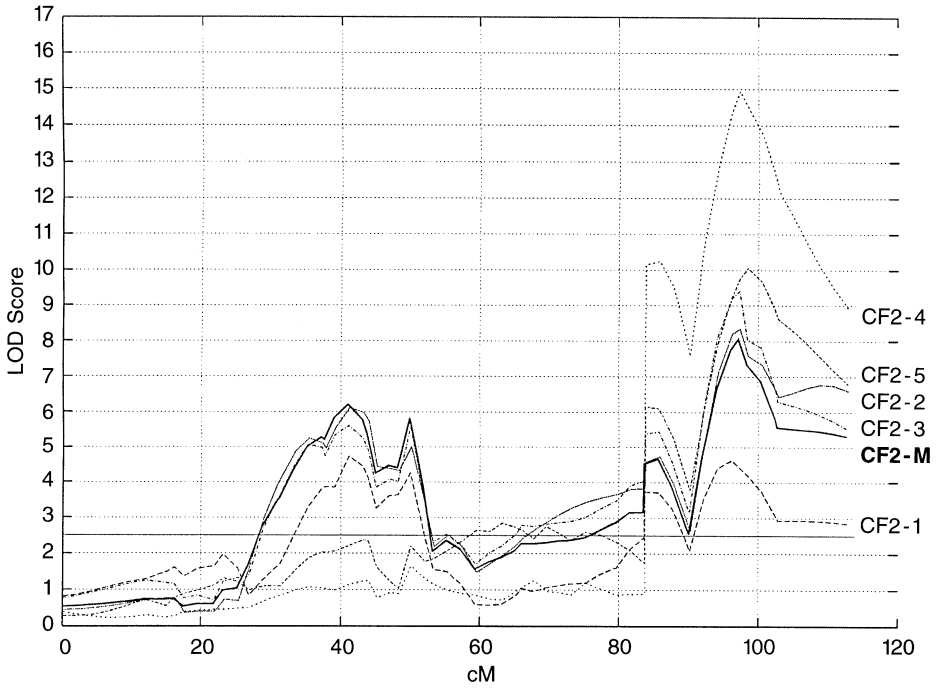

Figure 2 LOD scores of QTLs detected on chromosome 2. The *line* at LOD 2.5 represents the threshold for significance. Data were from F_2 families evaluated at different developmental stages (CF2-1 to CF2-5). CF2-M refers to QTL analysis carried out on mean CF2 data

method is that marker grouping does not depend only on thresholds of recombination frequencies or on degrees of linkage between markers. However, two limitations of this approach have become apparent: (1) not all mapped markers can be used for a new segregating population of a species; for example, out of 260 RFLP probes tested, only 80 were found to be polymorphic in the *K2* population; (2) not all markers mapped to a linkage group in one population may necessarily be assigned to the same position in a second population. In this study, conflicts arose; for example, for markers MP0038, placed by Barzen et al. (1995) on linkage group 1, and for marker KP1178, placed by Pillen et al. (1993) on linkage group 8. These two markers were finally mapped with high LOD scores to chromosomes 5 (MP0038) and 1 (KP1178) in the *K2* population. Chromosomal rearrangements in parental lines may account for such differences (Causse et al. 1996; details also in Schumacher et al. 1996). A more probable source of error, however, is the existence of duplicated DNA sequences in the genome of a crop, or the effect of non-canonical segregation ratios on linkage group and position assignments.

AFLP mapping was based on 16 primer combinations, which revealed 180 polymorphic fragments – on average almost 11 scorable bands per gel. Schondelmaier et al. (1996) have reported finding about 30 polymorphic bands per primer combination for their segregating sugar beet population. This evident difference may be due to the use of *Eco*RI instead of HIND ATA primers but could also result from a lower degree of DNA polymorphism in the K2 population. The integration of AFLPs into the linkage map did not present any particular problems because it was based on RFLP anchor probes. For eight out of nine linkage groups, the starting order could be derived by the MAPMAKER procedure 'order' using only RFLPs. For linkage group 1, which could not be defined using RFLP anchor markers, only 1 informative RFLP marker was available, and no starting order could be established automatically. This may indicate that the number of AFLP markers used in this study is insufficient for the assembly of a RFLP independent map. Problems in the construction of a linkage map for sugar beet based only on AFLPs have already been reported by Schondelmaier et al. (1996). The lower informational content of AFLPs compared to RFLPs is due to the dominant scoring of AFLPs, which precludes distinction between homozygously and heterozygously inherited, amplified fragments.

The clustering of markers observed in this study has also been reported in the mapping studies of sugar beet by Barzen et al. (1992, 1995), Pillen et al. (1992), Schondelmaier et al. (1996) and Nilsson et al. (1997), regardless of the type of marker used. Regions of clustering are located mostly in the centre of linkage groups, indicating that rates of recombination are lower in putative centromeric regions. Clustering was particularly evident in the study of Nilsson et al. (1997), but the number of marker loci mapped was more than twice that used in our study.

QTL analyses

In our experiments, single F_2 and TC plants of sugar beet infected by *C. beticola* showed a different responses. By using molecular markers, we were able to resolve this response at a high precision into the contribution of discrete units corresponding to QTLs allocated to specific linkage groups. No doubt remains as to the existence and significance of these genetic factors: their LOD scores are highly significant. *Cercospora* resistance was scored

at several independent times. This allowed us to monitor resistance reactions in several intra-seasonal environments, with a heritability of the trials of above 0.8 . The year 1996 was suitable for this type of approach due to the epidemiological conditions at the Italian test site. A test under artificial inoculation conditions was also included, restricted to 1 year and to a single location in Germany.

The results obtained are straightforward. In the case of QTL studies describing the polygenic inheritance of tolerance to diseases (see, for example, Leonardt-Schippers et al., 1995), QTLs involved in the response to infection in part appeared to differ when analysed at different stages of plant development. In our data, evidence for changes in the relevance of QTLs during the growth period is minimal. On the contrary, assignments of QTLs were quite stable, remaining essentially the same across time of year and modality of pathogen inoculation. Three major QTLs were found to be segregating in both the F_2 and TC progenies. They map on linkage groups 2, 6 and 9. All three are evident under both natural and artificial inoculation conditions. LOD scores for the existence of three QTLs were higher in F_2 families than in TC families. This is in agreement with the fact that the genetic variance of a population derived from parents that differ greatly in resistance to leaf spot disease will be reduced in a test cross derived from a common parent susceptible to *Cercospora*. Only QTLs with dominance effects are detectable in a TC. Consequently, a loss in resolving power for QTL analysis was expected. The lower phenotypic variance in TC progeny compared to the estimate for the F_2 clones supports this conclusion (Table 3).

A further three major QTLs were evident only from F2 data. These QTLs map on linkage groups 2, 4 and 5. It is highly possible that the QTL alleles concerned are recessive, even if other interpretations can be provided.

The finding, only in the TC families inoculated artificially, of a highly significant QTL at position 90 on linkage group 3 is not easy to explain. It must, however, be considered that the artificial inoculation can reveal large differences among genotypes due to a specific geneotype x inoculation interaction. The result has to be treated with caution because under field conditions this QTL was poorly visible (though it was manifest at early stages of plant growth; see CF2-1 and CTC-1).

The QTL analysis presented in Table 4, together with the clear definition of major QTLs, shows a certain degree of variability in the positioning of QTLs along the linkage groups to which they map, as was also found in other QTL mapping experiments (Schäfer-Pregl et al. 1998). These differences have been traced back to genotype x environment interactions (Asins et al. 1994; Freyre and Douches 1994). In the present study, intra-seasonal Genotype x Environment interactions could have caused some differences, particularly when the F_2 and TC series of estimates are considered. Differences between the two types of families were most probably due to the genetic nature of the QTLs involved, as already outlined.

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