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ORIGINAL PAPER

An anchored linkage map for sugar beet based on AFLP, SNP and RAPD markers and QTL mapping of a new source of resistance to *Beet necrotic yellow vein virus*

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Abstract Rhizomania, caused by *Beet necrotic yellow vein virus* (BNYVV), is an important sugar-beet disease worldwide and can result in severe losses of root yield and sugar content. We have identified a major QTL for BNYVV resistance from a new source in a segregating population of 158 individuals. The QTL explained an estimated 78% of the observed phenotypic variation and the gene conferring the partial resistance is referred to as *Rz4*. AFLP was used in combination with bulked segregant analysis (BSA) to develop markers linked to the resistance phenotype. AFLP marker analysis was extended to produce a linkage map that was resolved into nine linkage groups. These were anchored to the nine sugar-beet chromosomes using previously published SNP markers. This represents the first anchored sugar-beet linkage map to be published with non-anonymous markers. The final linkage map comprised 233 markers covering 497.2 cM, with an average interval between markers of 2.1 cM. The *Rz4* QTL and an *Rz1* RAPD marker were mapped to chromosome III, the known location of the previously identified BNYVV resistance genes *Rz1*, *Rz2* and *Rz3*. The availability to breeders of new resistance sources such as *Rz4* increases the potential for breeding durable disease resistance.

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Introduction

Sugar beet (*Beta vulgaris* subsp. *vulgaris*) is a member of the family *Amaranthaceae* (formerly *Chenopodiaceae*), which also contains the crop species spinach (*Spinacea oleracea* L.) and quinoa (*Chenopodium quinoa*). It is diploid with $2n = 18$ chromosomes and a haploid genome size of 758 Mb (Arumuganathan and Earle 1991). Cultivated forms of the species, including fodder beets, leaf beets and garden beets, are sexually compatible with wild sea beet (*B. vulgaris* subsp. *maritima*). The species is outbreeding and highly heterozygous with a multi-allelic gametophytic self-incompatibility system.

Sugar beet is susceptible to diseases which impact on the sugar yield. Rhizomania is an important sugar-beet disease worldwide and can result in severe losses of root yield and sugar content (Johansson 1985). It is caused by *Beet necrotic yellow vein virus* (BNYVV) which is transmitted by the obligate root parasite *Polymyxa betae* Keskin. The disease was first described in Italy in the 1950s (Canova 1959) but was only detected for the first time in the UK in 1987 (Hill and Torrance 1989) where it has since been recorded mainly on the light, sandy soils of East Anglia (Asher and Dewar 2001). It has been found in Asia, the USA, southern and central Europe and Scandinavia (Asher 1993; Tamada 1999; Lennefors et al. 2000) and is expected to continue its spread worldwide (Richard-Molard and Cariolle 2001; Rush et al. 2006). Partial resistance to BNYVV was discovered in the USA in a breeding line developed by the Holly Sugar Company and was found to be conditioned by a single dominant gene (*Rz1*) (Lewellen et al. 1987; Scholten et al. 1996). The 'Holly' source of resistance is now widely deployed in

sugar-beet cultivars throughout Europe and the USA, although it may be inadequate under severe disease pressure (Paul et al. 1993; Lewellen 1995; Rush et al. 2006). Resistance to BNYVV has also been found in *Beta vulgaris* subsp. *maritima* accessions WB42 (*Rz2*) and WB41 (*Rz3*) originating in Denmark (Lewellen et al. 1987). The level of resistance conditioned by the dominant *Rz2* gene appears to be higher than that conditioned by *Rz1* (Paul et al. 1993).

Genetic mapping is an important tool for analysing the genetic basis of traits and, if markers tightly linked to the trait of interest are identified, also provides a means of tagging the inheritance of traits in marker-assisted selection (reviewed by Francia et al. 2005). Genetic mapping in sugar beet has recently been reviewed by Jung (2004). Several linkage maps have been produced that incorporate RFLP, AFLP and SSR markers (e.g. Schondelmaier et al. 1996; Rae et al. 2000) but these have been developed collaboratively with private breeding companies and the markers are anonymous. Uphoff and Wricke (1995) and Nilsson et al. (1997) developed maps incorporating 85 and 160 RAPD markers respectively. Although these markers are publicly available, the linkage groups were not anchored to chromosomes and cannot be correlated with those of other linkage maps. This limits the potential uses of the maps for trait identification in different mapping pedigrees and for marker-assisted selection. Also, RAPD markers are treated with reserve due to frequently observed problems with reproducibility (e.g. Ellsworth et al. 1993; Pérez et al. 1998).

From previous efforts to map BNYVV resistance genes in sugar beet, it is not clear whether *Rz2* is allelic with either *Rz1* or *Rz3* or closely linked on chromosome III. However, the fact that WB41 and WB42 were both collected in Denmark at around the same time suggests that perhaps *Rz2* and *Rz3* are the same. Scholten et al. (1999) and Amiri et al. (2003) estimated the genetic distance between *Rz1* and *Rz2* to be 20 cM and 35 cM respectively. However, in these studies, individual plants were classified as either resistant or susceptible whereas the phenotypic distribution was clearly quantitative, an approach that is likely to result in the overestimation of recombination frequency (Gygax et al. 2004). Gidner et al. (2005) carried out QTL mapping of BNYVV resistance from WB41 and found the distance between *Rz1* and *Rz3* to be 5 cM in a population of ~270 individuals.

The availability to breeders of new resistance sources increases the potential for breeding durable disease resistance. We report here the first anchored sugar-beet linkage map to be published with non-anonymous markers and a new source of resistance to BNYVV which we refer to as *Rz4*. This linkage map forms the basis of a longer-term programme to identify and map new sources of resistance to important sugar-beet diseases from *Beta* germplasm.

Materials and methods

Plant material

A BNYVV resistant hybrid plant was crossed to a susceptible male-sterile plant of the breeding line CALE to produce the full-sib R36 mapping population consisting of 158 individuals. The resistant hybrid parent was derived from a cross between a selected resistant plant of line R36 and one plant of breeding line CALE. Line R36 segregates for BNYVV resistance and is derived from C50 (Lewellen and Whitney 1993), a composite cross of about 60 *Beta vulgaris* subsp. *maritima* accessions with sugar beet. Genetic male-sterility, conferred by the single recessive gene a_1 (Owen 1952), was used to ensure the production of hybrid seed. In accordance with prevailing UK Plant Health regulations, known resistant plants, that had low virus titres whilst being grown in BNYVV-infested soil, could not be removed from the rhizomania glasshouse and used for crossing. Therefore the breeding programme was based on progeny selection. Small sample populations from hybrid progeny were tested for the inheritance of resistance in glasshouse BNYVV detection tests as described below. Seeds of line R36 (Lewellen 1997) were obtained from R. T. Lewellen, USDA-ARS. Seeds of line CALE were obtained from Lion Seeds Ltd, Maldon, Essex, UK.

BNYVV detection

Seeds were sown in soil collected at Blaxhall, Suffolk, UK that was naturally infested with the B type strain of BNYVV as determined by RT-PCR. Plants were grown for 6 weeks in a glasshouse under optimal conditions for root infection and virus multiplication. A triple antibody sandwich ELISA test was carried out on sap extracted from the roots to provide a quantitative estimate of the virus titre in each plant. Full details of the methods used to infect plants and measure virus content are described by Asher et al. (2002).

DNA extraction

Genomic DNA was extracted from snap-frozen leaf tissue of the R36 mapping population individuals and parents using the Nucleon Phytopure Plant DNA

Extraction Kit (Amersham Biosciences) with the addition of 10 mM 2-mercaptoethanol to Reagent 1. After cooling samples on ice, DNA was isolated by phenol:chloroform:isoamylalcohol extraction and isopropanol precipitation (Sambrook et al. 1989) then dissolved in 50 µl sterile distilled water (SDW). RNA was degraded by addition of 1 µg RNase (Roche) and incubation for 15 min at 37°C. The quantity and quality of DNA were assessed by agarose-gel electrophoresis using 0.8% agarose with known concentrations of uncut lambda DNA (Roche). Gel images were captured using the GeneGenius gel documentation system with GeneSnap software (Syngene). DNA concentrations were calculated using GeneTools software (Syngene).

Fluorescent AFLP protocol

AFLP reactions were carried out essentially as described by Vos et al. (1995). Genomic DNA (125 ng) was digested for 1 h at 37°C in a 15 µl volume containing 2.5 U *Eco*RI and 1X One-Phor-All Buffer PLUS (Amersham Biosciences) with 2.5 U *Mse*I and 1.5 µg bovine serum albumin (New England Biolabs). For ligation, 5 µl of solution containing 0.5 µM *Eco*RI adapter (5′-CTCGTAGACTGCGTACC-3′; 3′-CTGACGCATGGTTAA-5′) and 5 µM *Mse*I adapter (5′-GACGATGAGTCCTGAG-3′; 3′-TACTCAGGACTCAT-5′) with 1 U T4 DNA ligase, 1 mM ATP and 1X One-Phor-All Buffer PLUS (Amersham Biosciences) was added to the digest and incubated at 37°C for 5 h. Pre-amplification was performed in a 5 µl volume containing 1.25 µl 10-fold diluted digested-ligated DNA, 1X Promega Master Mix, 0.5 µM *Eco*RI primer (5′-GTAGACTGCGTACCAATTC-3′) and 0.5 µM *Mse*I primer (5′-GACGATGAGTCCTGAGTAA-3′), each with an additional 3′ selective nucleotide. Thermocycling conditions were as follows: 5 min at 65°C followed by 30 cycles of 30 s at 94°C, 30 s at 56°C and 60 s at 72°C. All thermocycling was performed using a PCR Express Thermal Cycler (ThermoHybaid). Fluorescent, multiplex selective amplification was performed in a 5 µl volume containing 1 µl 20-fold diluted pre-amplified DNA, 1X Qiagen Multiplex PCR Master Mix, 0.7 µM FAM™, NED™ and VIC®—labelled *Eco*RI selective primers (5′-GACTGCGTACCAATTC-3′) and 1.4 µM *Mse*I selective primer (5′-GATGAGTCCTGAGTAA-3′), each with three additional 3′ selective nucleotides. Amplification was also performed with 1X Promega Master Mix instead of 1X Qiagen Multiplex PCR Master Mix. Thermocycling conditions were as follows: 95°C for 15 min; 13 cycles of 30 s at 94°C, 30 s at 65°C with a 0.7°C reduction per cycle and 120 s at 72°C. The program continued with 25 cycles of 30 s at 94°C, 90 s at 55°C and 90 s at 72°C followed by a final extension of 10 min at 72°C. One µl 20-fold diluted selective PCR product was mixed with 0.2 µl GeneScan™–500 LIZ™ size standard and 8.8 µl Hi-Di Formamide (Applied Biosystems), denatured at 95°C for 5 min and placed on ice. AFLP fragments were separated on an ABI Prism 3100 Genetic Analyzer (Applied Biosystems) using POP-6™ polymer and 36 cM arrays. GeneScan® 3.7 software (Applied Biosystems) was used to size the fragments based on the internal lane standard and Genotyper® 3.7 software (Applied Biosystems) used for scoring the data. Polymorphic bands were named according to the selective primers used to amplify them and the size (in base pairs) of the fragments scored.

Bulked segregant analysis

Bulked segregant analysis (BSA) was performed, as described by Michelmore et al. (1991), in order to identify markers co-segregating with BNYVV resistance. The diluted DNA samples (10 ng/µl) of the ten most resistant (*R*) and ten most susceptible (*S*) individuals of the R36 mapping population were pooled into *R* and *S* bulks that were screened with 240 AFLP primer combinations. The mean ELISA values of the *R* and *S* individuals were 0.247 and 2.657 respectively. The primer combinations that gave the most number of polymorphic bands between the bulks were tested on 7*R* and 7*S* bulk individuals to eliminate false positive markers prior to screening against the whole population. In addition, 12 randomly chosen AFLP primer combinations were used to generate further AFLP markers for map construction.

SNP genotyping

Anchoring SNP genotyping was performed essentially as described by Möhring et al. (2004) but with minor adaptations. The linkage groups presented by these authors were assigned to chromosomes according to Butterfass (1964) and Schondelmaier and Jung (1997). PCR primer and extension primer core sequences were as described, but after initial tests only three successful markers were used per multiplex/linkage group (LG). Multiplex PCR amplification was performed in a 5 µl volume containing 10 ng genomic DNA, 200 µM dNTPs (Promega) and 0.1 µM each primer with 1X PCR Buffer, 1.5 mM $MgCl_2$ and 0.025 U HotStarTaq® DNA Polymerase (Qiagen). All thermocycling was performed using a PCR Express Thermal Cycler (ThermoHybaid). Two µl of PCR product were

purified with 0.8 µl ExoSAP-IT (Amersham Biosciences) according to the protocol provided. Primer extension was performed in a 5 µl volume containing 1.5 µl purified PCR product, 0.25 µM each extension primer and 0.25 µl SNaPshot Multiplex Ready Reaction Mix (Applied Biosystems). The first, second and third extension primers used from each LG (Möhring et al. 2004) had sizes of 24, 30 and 36 nucleotides respectively and were not HPLC-purified. Three µl of extension reaction product were purified with 0.5 U calf intestinal phosphatase (New England Biolabs) according to the protocol provided. Prior to separating fragments, 0.75 µl purified sample were mixed with 0.25 µl GeneScan™–120 LIZ™ size standard and 9 µl Hi-Di Formamide (applied biosystems), denatured at 95°C for 5 min and placed on ice. Fragments were separated, sized and scored as described in the fluorescent AFLP protocol.

RAPD protocol

PCR was carried out in a 10 µl volume containing 20 ng genomic DNA, 0.4 µM RAPD primer and 1X Qiagen Multiplex PCR Master Mix. Thermocycling conditions were as follows: 15 min at 95°C followed by 45 cycles of 60 s at 94°C, 90 s at 35°C and 120 s at 72°C followed by a final extension of 5 min at 72°C. Thermocycling was performed using a PCR Express Thermal Cycler (ThermoHybaid). The RAPD fragments were separated by electrophoresis through 1.4% agarose. Gel images were captured using the GeneGenius gel documentation system with GeneSnap software (Syngene). The primers tested for the rhizomania resistance (*Rz1*) locus were OPF6, OPN9, OPD18, OPP13, OPB4, OPAN7, OPT20, OPH13, OPAS7 and OPC15 (Barzen et al. 1997).

Construction of a linkage map of the R36 resistance source

SNP, RAPD and AFLP markers were screened against 30, 48 and 158 individuals of the R36 mapping population respectively, along with the parents. Segregation codes were assigned to markers as follows: *abxaa* for dominant markers segregating in the resistant male parent only and *abxab* for co-dominant SNP markers segregating in both parents. Data analysis was performed using JoinMap version 3.0 software (Van Ooijen and Voorrips 2001) that uses the estimation procedures for cross-pollinators as described by Maliepaard et al. (1997). The software was used to test markers for segregation distortion using a chi-square test. The R36 linkage map was constructed using all markers of type *abxaa*. For the purpose of anchoring the linkage groups, SNP markers of type *abxab* were included. Linkage groups were determined using a minimum LOD threshold of 3.0 and map construction performed using the Kosambi mapping function with the following JoinMap parameter settings: Rec = 0.4, LOD = 1.0, Jump = 5. A third round of ordering whereby problematic markers are forced onto a map was not employed; such markers were discarded. Resulting linkage maps were drawn using MapChart software (Voorrips 2001).

QTL mapping

All segregating AFLP markers were tested for significant association with BNYVV resistance using the non-parametric rank sum test of Kruskal-Wallis (Lehmann 1975) found in MapQTL® software 4.0 (Van Ooijen et al. 2002). For each individual test the suggested significance level (*P*-value) of 0.005 was used in order to obtain an overall significance level of about 0.05. The estimated proportion of phenotypic variation (R^2) explained by marker alleles was calculated as 1—(sum of squares of marker classes/total sum of squares).

Results

BNYVV resistance scores

The distribution of BNYVV ELISA values for the R36 mapping population is shown in Fig. 1 and suggests the segregation of a QTL with a major effect on resistance to BNYVV. The mean ELISA value of 10 uninfected control plants was 0.12 and ELISA values of the infected individuals ranged from 0.147 to 2.796.

Molecular markers

A total of 243 scorable polymorphic AFLP markers of type *abxaa* were identified from 36 primer combinations. Analysis of the genotype frequencies of these markers revealed that 25% showed segregation distortion ($P \leq 0.1$: chi-square test).

Of the 27 SNP markers tested (3 per linkage group) from the full set of 52 described by Möhring et al. (2004), 9 were found to be monomorphic. These were: MPatp3, MPcab4, MPinvcw, MP0143, MPcab5, MP_sht, MPpgdh, MP7M20 and MP2G14. Of the remaining 18 which were used for map construction, 16 were *abxaa* and 2 were *abxab* (MP0075 and MP_sps). Of the ten RAPD primers tested for the *Rz1* locus only OPC15 produced a segregating band

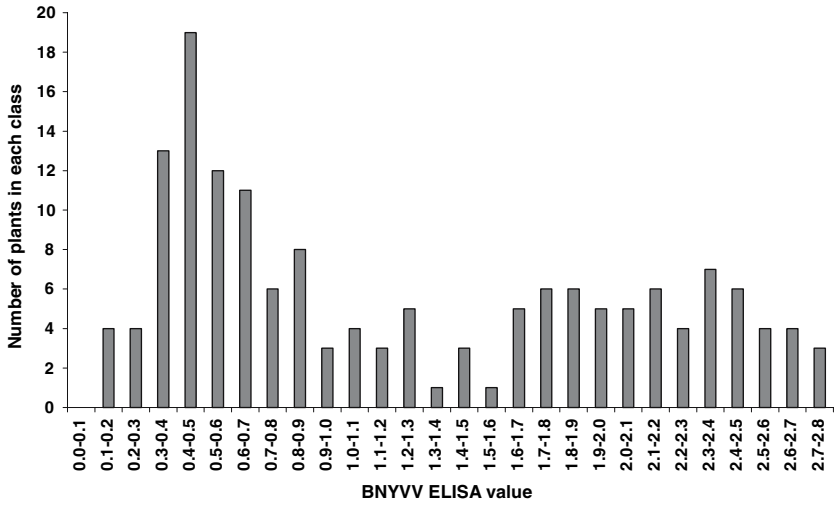

Fig. 1 Distribution of BNYVV levels in the roots of plants from the R36 mapping population

of the correct size (marker OPC15-1800). This marker was of type *abxaa* and showed no segregation distortion. One SNP marker (MP0176) showed segregation distortion.

Construction of a linkage map of the R36 resistance source

For the R36 linkage map, markers were assigned to 9 linkage groups at a LOD threshold of 3.0. When the LOD threshold was increased to 4.0 the linkage groups remained the same except for the loss of three markers (MP_pS2, EACA/MTCG-252 and MP0075) from groups I, IV and VII respectively. At LOD 3.0 only marker MP_sps (chromosome VIII) was not assigned to a group. The final R36 linkage map (Fig. 2) comprised 215 AFLP, 17 SNP and 1 RAPD markers (233 in total) covering 497.2 cM, with an average interval between markers of 2.1 cM. Each of the nine linkage groups had at least one chromosome-anchoring SNP marker.

QTL mapping

Kruskal–Wallis analysis of all segregating AFLP markers revealed that only the markers on chromosome III were significantly associated with BNYVV resistance using the suggested threshold of $P = 0.005$. The chromosome III AFLP markers were all significantly associated with BNYVV resistance at $P \leq 0.0001$ (Table 1), indicating the presence of a segregating QTL. The marker with the highest test statistic (EACA/MCAG-179) explained an estimated 78% of the phenotypic variation (R^2). The two markers with the lowest test statistic were located at either end of chromosome III. The mean ELISA values of the genotypic classes with and without marker EACA/MCAG-179 were 0.63 (SD = ±0.34) and 2.07 (SD = ±0.43) respectively.

Discussion

Through construction of a linkage map and use of bulked segregant analysis (BSA) in sugar beet we have successfully identified a QTL having a major effect on resistance to *Beet necrotic yellow vein virus*. Marker analysis and linkage mapping were achieved using a combination of AFLP, SNP and RAPD approaches.

Phenotypic analysis of R36 mapping population

The distribution of BNYVV ELISA values in the R36 mapping population suggests the segregation of a QTL with a major effect on resistance to BNYVV. The phenotypic overlap between resistant and susceptible classes resembles those seen in populations segregating for *Rz1*, *Rz2* and *Rz3* (Scholten et al. 1996; Gidner et al. 2005). Gidner et al. (2005) found that the phenotypic variation observed in plants heterozygous for *Rz3* was explained by environmental rather than genetic factors. In glasshouse tests *Rz3rz3* plants with a low virus content, as well as those with a high virus content, gave progenies with virus contents ranging from low to high. The ELISA value of the most resistant individual from the R36 mapping population was not as low as the mean value of the uninfected control plants, suggesting that the QTL confers only partial resistance to BNYVV. Individuals carrying *Rz1*, *Rz2* and *Rz3* also show partial resistance to BNYVV (Scholten et al. 1996; Amiri et al. 2003; Gidner et al. 2005). The similarity in phenotype conferred by the four different

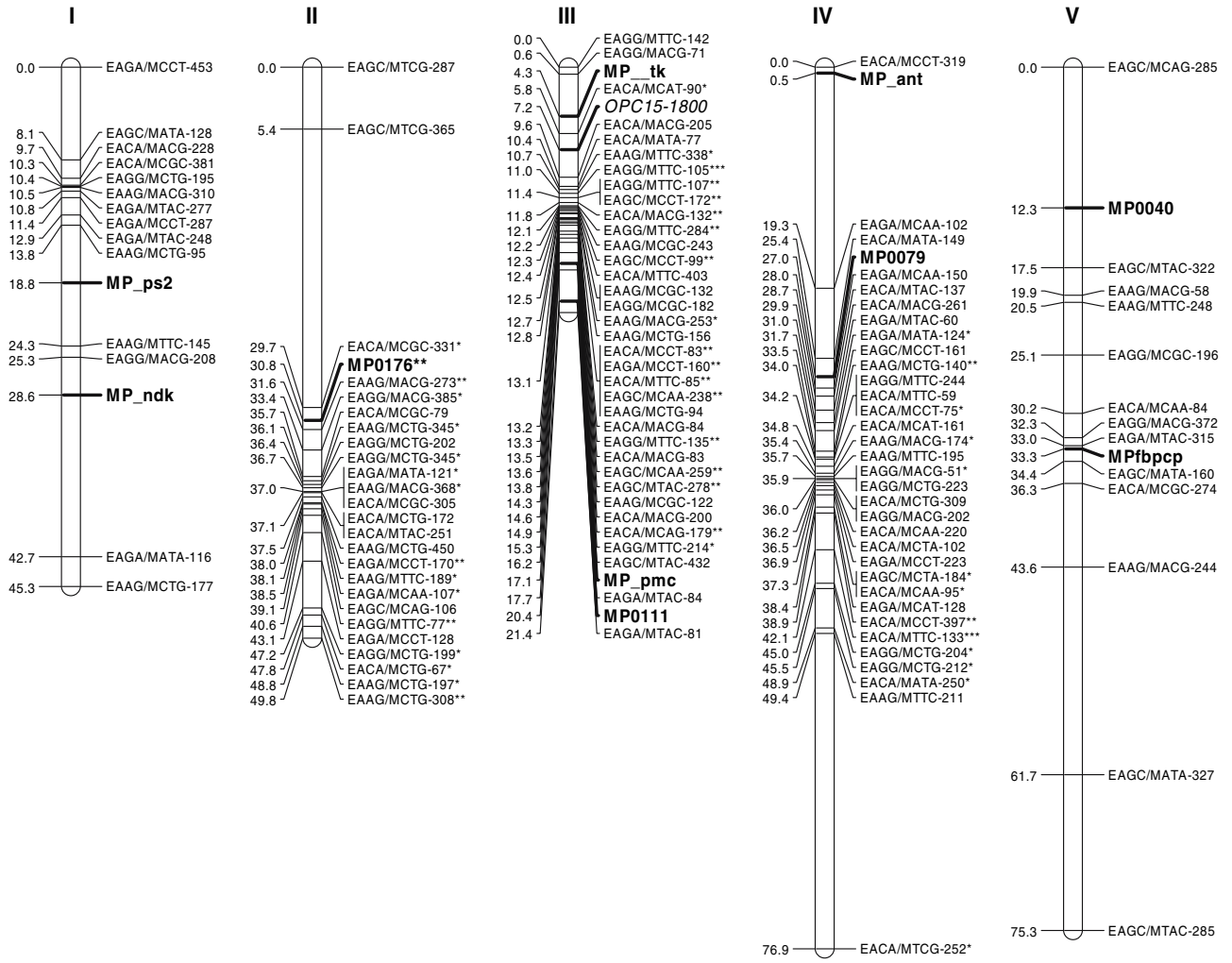

Fig. 2 R36 linkage map of sugar beet. Cumulative distances are in centiMorgans and are indicated on the *left side* of the linkage groups. SNP markers are in *bold*. RAPD markers are in *italics*. All other markers are AFLP markers; details of their nomenclature are described in "Materials and methods". Markers showing segregation distortion are indicated by *asterisks* (* $P < 0.1$, ** $P < 0.05$, *** $P < 0.01$)

sources suggests that similar resistance mechanisms may operate in each case.

AFLP marker identification and segregation analysis

The application of BSA to the R36 mapping population resulted in the identification of a large number of AFLP markers linked to the BNYVV partial resistance phenotype. BSA using AFLP markers has been widely employed to map a number of important resistance genes in plants (e.g. Asnaghi et al. 2004; Bakker et al. 2004). A high proportion (25% at $P \leq 0.1$) of the AFLP markers identified here showed segregation distortion. This is comparable to proportions of 15.5 and 19.8% at $P \leq 0.05$ (Schumacher et al. 1997) and 15% at $P \leq 0.01$ (Pillen et al. 1993) previously reported for sugar beet. Of 284 AFLP markers tested in the related crop species quinoa, only 4.9% showed segregation distortion at $P \leq 0.05$ (Maughan et al. 2004). Markers can show distorted segregation because of a number of genetic, physiological and/or environmental factors (Xu et al. 1997) and distortion can occur due to linkage to a gene affecting viability (e.g. Cervera et al. 2001). Inclusion of such markers may affect the accuracy for determining the order of markers (Lorieux et al. 1995) but they are commonly retained in genetic maps so as not to deplete them of regions of potential interest.

Clusters of markers showing segregation distortion were found spread along chromosomes II, III and IV. Both Pillen et al. (1993) and Uphoff and Wricke (1995) reported clustering of markers with distorted segregation ratios at the termini of sugar-beet linkage groups. This variation may be due to the different pedigrees

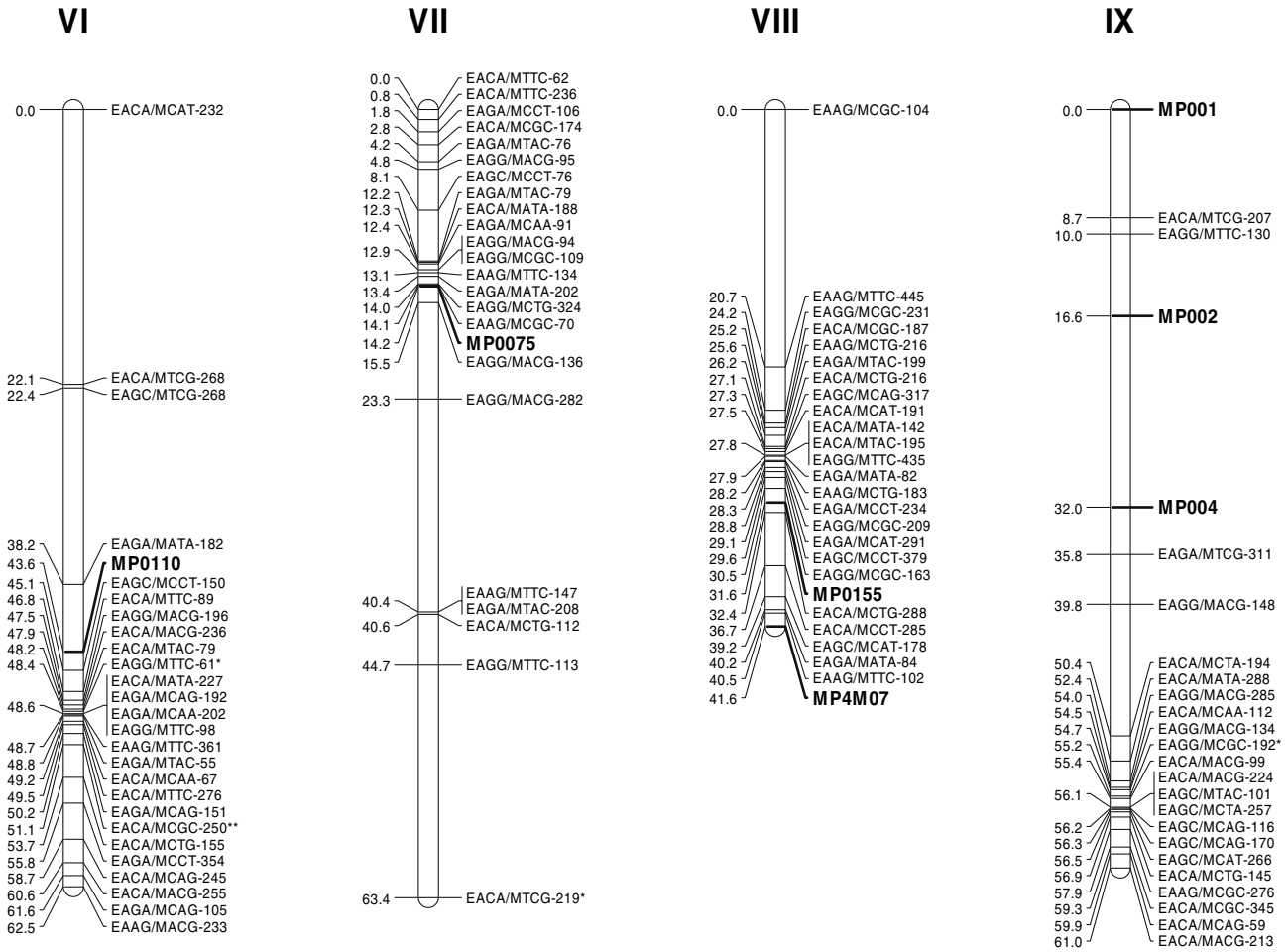

Fig. 2 continued

used and subsequent selection pressures in forming the different mapping populations. Wagner et al. (1992) found that specific sugar-beet isozyme and morphological marker loci only displayed distorted segregation ratios in certain progeny families. Within the regions of marker segregation distortion reported here were individual markers with a 1:1 segregation ratio. This phenomenon has been reported elsewhere in plant linkage mapping (e.g. Hanley et al. 2002; Lowe and Walker 2006). Possible explanations for this occurrence are variations between neighbouring markers in the number of informative individuals analysed (see Table 1) and superimposing of marker loci of equivalent electrophoretic mobility.

SNP and RAPD marker analysis

The SNP marker set and a modified version of the multiplexed detection protocol described by Möhring et al. (2004) were successfully applied here to anchor the AFLP linkage groups to chromosomes. The most important alteration to the protocol was that desalted rather than HPLC-purified extension primers were used, saving considerably on expense. We found little difference when using extension primers up to 36 bp purified by these two methods. Of the 27 SNP markers tested 18 were found to be polymorphic, suggesting that this marker set is widely applicable to genetic mapping and fingerprinting in sugar beet. Of ten markers tested using RAPD primers only one was found to be present and polymorphic in this mapping population. It is not clear whether this low number, in comparison with the number of SNP markers utilised, is due to the reported low reproducibility of RAPD fragments (e.g. Ellsworth et al. 1993; Pérez et al. 1998) or due to genetic variation altering the RAPD profile.

R36 linkage map

The AFLP markers identified were resolved into a map with nine linkage groups, the haploid chromosome number of sugar beet, and these groups were

Table 1 Results of Kruskal–Wallis analysis of AFLP markers on chromosome III to identify significant marker-trait associations

Marker	Position (cM)	nr inf	K^*
EAGG/MTTC-142	0.0	125	51.270
EAGG/MACG-71	0.6	137	61.988
EACA/MCAT-90	5.8	144	62.026
EACA/MACG-205	9.6	136	93.447
EACA/MATA-77	10.4	133	88.486
EAAG/MTTC-338	10.7	124	79.467
EAGG/MTTC-105	11.0	124	66.096
EAGG/MTTC-107	11.4	125	68.953
EAGC/MCCT-172	11.4	132	74.311
EACA/MACG-132	11.8	137	83.538
EAGG/MTTC-284	12.1	124	73.604
EAAG/MCGC-243	12.2	116	78.785
EAGC/MCCT-99	12.3	132	78.065
EACA/MTTC-403	12.4	122	71.798
EAAG/MCGC-132	12.5	115	75.281
EAGG/MCGC-182	12.5	113	74.481
EAAG/MACG-253	12.7	136	78.692
EAAG/MCTG-156	12.8	130	75.695
EACA/MCCT-83	13.1	132	81.204
EAGA/MCCT-160	13.1	131	80.057
EACA/MTTC-85	13.1	125	75.702
EAGC/MCAA-238	13.1	147	92.706
EAAG/MCTG-94	13.1	134	79.512
EACA/MACG-84	13.2	136	76.777
EAGG/MTTC-135	13.3	125	71.255
EACA/MACG-83	13.5	136	81.199
EAGC/MCAA-259	13.6	147	88.228
EAGC/MTAC-278	13.8	122	73.583
EAAG/MCGC-122	14.3	117	80.629
EACA/MACG-200	14.6	136	76.700
EACA/MCAG-179	14.9	148	100.509
EAGG/MTTC-214	15.3	125	76.128
EAGC/MTAC-432	16.2	121	71.508
EAGA/MTAC-84	17.7	121	69.743
EAGA/MTAC-81	21.4	121	50.303

Map positions are in centiMorgans.

nr inf The number of informative individuals (i.e. with both genotypic and phenotypic data) and K^* the Kruskal–Wallis test statistic. All markers were significant for association with the phenotype at $P \leq 0.0001$

successfully anchored to chromosomes using SNP markers developed by Möhring et al. (2004). Analysis of these SNP markers shows that marker order is retained between maps and suggests that they map to the same locations in terms of putative centromeric clusters and sub-telomeric regions. In addition to the AFLP and SNP markers, a RAPD marker for the BNYVV resistance gene *Rz1* was mapped. This marker was originally located at ~6 cM (Barzen et al. 1997) from the gene and mapped to the correct chromosome here.

The clustering of AFLP markers on linkage groups as found here is a common phenomenon in a wide range of plant species (e.g. Hanley et al. 2002; Sugita et al. 2005). Schondelmaier et al. (1996) incorporated AFLP markers into a linkage map of sugar beet and found a similar pattern of dense clustering. Such clusters may be explained by reduced recombination in chromosomal regions such as centromeres (Tanksley et al. 1992; AlonsoBlanco et al. 1998). The R36 linkage map we present here represents the first anchored sugar-beet map to be published with non-anonymous markers. It forms the basis of a longer-term programme to identify and map new sources of resistance to sugar-beet diseases from *Beta* germplasm and is a useful and important resource for sugar-beet research. The DNA samples from the mapping population are available to researchers.

QTL mapping of *Rz4*

A major QTL for BNYVV resistance explaining an estimated 78% of the phenotypic variation was located to chromosome III. The previously identified BNYVV resistance genes, *Rz1*, *Rz2* and *Rz3*, are also found on this chromosome. We refer to the gene conferring BNYVV resistance in the R36 mapping population as *Rz4*, although the presence of more than one segregating resistance gene on chromosome III is not ruled out. After initial analysis of *Rz2* segregation, Scholten et al. (1996) suggested that the conferred BNYVV resistance was based on either one (or more) dominant major gene(s) showing distorted segregation, or two complementary dominant genes, both of which were required for resistance. After further analysis the latter hypothesis was rejected (Scholten et al. 1997). The distorted segregation effect observed for *Rz4* markers could be similar to that observed for *Rz2*. Further investigations are required to elucidate whether *Rz4* is a new BNYVV resistance gene or is allelic with *Rz1*, *Rz2* or *Rz3*. The markers identified in this study are potentially a valuable tool in achieving this by defining recombination events between *Rz* genes or by associating with specific *Rz* alleles.

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