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An extended map of the sugar beet genome containing RFLP and RAPD loci

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Abstract An updated map of sugar beet (*Beta vulgaris* L. ssp. *vulgaris* var 'altissima Doell') is presented. In this genetic map we have combined 248 RFLP and 50 RAPD loci. Including the loci for rhizomania resistance *Rz1*, hypocotyl colour *R* and the locus controlling the monogerm character *M*, 301 loci have now been mapped to the nine linkage groups covering 815 cM. In addition, the karyotype of some of the *Beta vulgaris* chromosomes has been correlated with existing RFLP and RAPD linkage maps.

Key words Sugar beet · *Beta vulgaris* · RFLP · RAPD

Introduction

Restriction fragment length polymorphism (RFLP) maps of sugar beet (*Beta vulgaris*) have recently been published (Barzen et al. 1992; Pillen et al. 1992, 1993). The map presented earlier by our group was based on 111 polymorphic RFLP loci and had a total length of 540 cM. It included the map positions of the genes *M* (polygerm vs. monogerm), *R* (hypocotyl colour) and *Rz1* (rhizomania resistance). In this paper we present an updated map that now covers 815 cM and is based on 301 loci distributed over all nine *Beta* chromosomes. The loci mapped have been revealed by RFLP probes and by randomly amplified polymorphic DNAs (RAPDs; Williams et al. 1990). By considering the existing cytogenetical studies (Pillen et al. 1992, 1993; Wagner et al. 1992; Jung et al. 1992; Barzen et al. 1992; Lange et al. 1993; Uphoff and Wricke 1992; Salentijn et al. 1992; Jung and Herrman 1991; Jung et al. 1990; Nakamura et al. 1991), we have correlated the karyotype of some of the *Beta vulgaris* chromosomes with RFLP/RAPD linkage maps already in existence.

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Materials and methods

The mapping population, consisting of 49 plants produced from the cross between 2 F_1 plants (P1 and P2), was previously described by Barzen et al. (1992). The plants were maintained both vegetatively and by selfing to obtain sufficient leaf material for DNA extraction, RFLP and RAPD analysis. The plants were propagated by the KWS Kleinwanzlebener Saatzucht AG Einbeck, Germany.

Methods used for the preparation of random genomic probes, DNA isolation, restriction digests, electrophoresis, blotting and hybridization were done as described by Barzen et al. (1992).

Four hundred and forty random 10-mer primers from Operon Technologies (Alameda, Calif., USA) were tested. Amplification reactions were performed in a reaction volume of 25 µl containing: 20 mM TRIS-Cl, pH 8.4; 50 mM KCl; 4 mM $MgCl_2$; 200 µM each of dATP, dCTP, dGTP and dTTP (Pharmacia); 0.2 µM primer; 25 ng genomic DNA; 1.5 units of Taq DNA polymerase (Gibco BRL); and overlaid with 2 drops of mineral oil. The amplification was performed in a Biometra DNA Thermal Cycler (Göttingen, Germany) programmed for 45 cycles, each one consisting of: 1 min at 92°C; 1.5 min at 35°C; and 2 min at 72°C. After the last cycle, the samples were incubated for 5 min at 72°C and then kept at 20°C. Amplification products were analyzed by electrophoresis in 1.4% agarose gels, detected by staining with ethidium bromide and photographed under UV light.

Linkage analysis was performed as described by Ritter et al. (1990). Each RFLP or RAPD fragment was scored as present or absent for all genotypes. Pairs of fragments defining two polymorphic loci (A and B) were considered, and the allelic configuration at these loci was determined by observing whether the two fragments were present in both parents (configuration AB/AB) or in only one parent (AB/00). Recombination frequencies were calculated by the 'maximum likelihood' method, and linkage groups were established by the 'nearest neighbor' method. Linkage subgroups were joined by considering loci with allelic fragments defining total linkage in repulsion (Ritter et al. 1990).

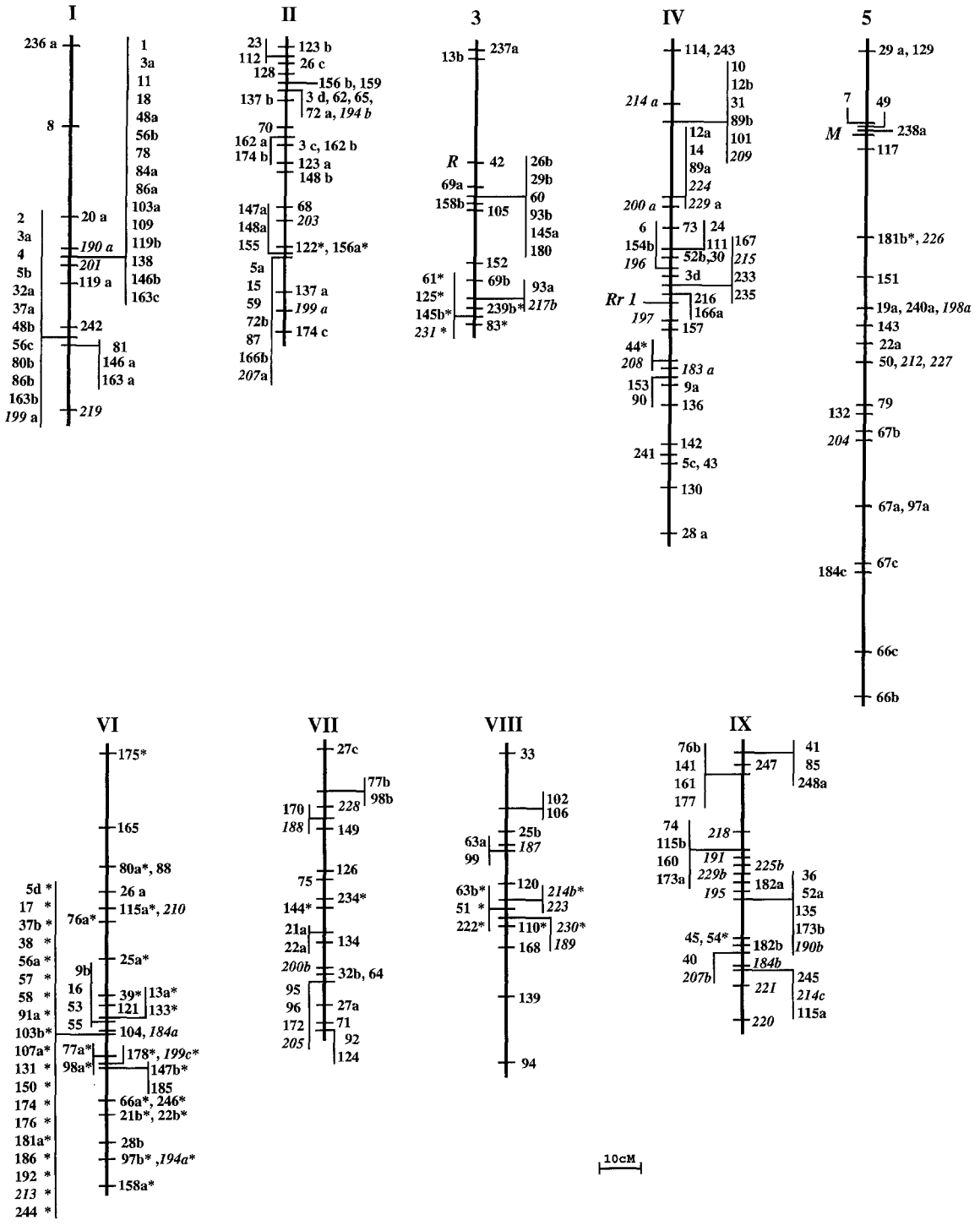

Fig. 1 Linkage map of sugar beet including RFLP and RAPD loci. The linkage groups labelled with *arabic numerals* are those that could be assigned to specific chromosomes. Loci with the *same number* but followed by *different letters* were revealed by the same genomic probe or primer. Map distances are given in centiMorgans. RFLP loci are given in *normal type* and RAPD loci in *italics*. *Rr1* is the rhizomania resistance locus, *R* is the locus controlling hypocotyl colour and *M* is the locus controlling the monogerm/polygerm seed type. Loci showing a distorted segregation are indicated by *asterisks*

Results and discussion

An additional 165 random sugar beet genomic clones have been used to map 140 new RFLP loci on the existing RFLP map consisting of 111 loci (Barzen et al. 1992). By using the RAPD technique we were able to add an additional 50 loci. A total of 301 loci have now been mapped covering 815 cM [Fig. 1; units as defined by Kosambi (1944)].

Different DNA, primer, Mg^{2+} and Taq DNA Polymerase concentrations were tested to establish a reproducible

polymerase chain reaction (PCR) protocol for RAPD analysis in sugar beet. DNA concentrations of 7.5–50 µg were tested in reaction volumes of 25 or 50 µl. DNA, primer and enzyme concentrations had no evident effect on the number and position of the PCR-amplified bands. The use of 4 mM Mg^{2+} resulted in clearer amplification patterns than those observed with lower concentrations. According to our data, the optimal PCR reaction conditions for sugar beet are those reported in the Materials and methods.

Out of the 440 RAPD primers screened with DNA from the two parents of our mapping population, 20% gave no amplification product; the rest yielded 1–10 bands in a size range of 100–2000 bp, of which 46% revealed no polymorphism between the parental DNAs, while 34% produced 1–3 polymorphic bands. Primers revealing a polymorphism were used for mapping. Forty-one RAPD loci segregated in the mapping population as dominant markers, predominantly with a segregation ratio of 1:1. At 9 loci a distorted segregation was observed. All 50 loci could be placed on the RFLP map (Fig. 1).

The position of some loci defined by RAPDs was checked by RFLP analysis. Polymorphic amplified DNA fragments were excised from the agarose gel, labelled with [^{32}P] and used as hybridization probes. In most cases the RFLP pattern obtained was quite complex, suggesting that the probe revealed the presence of repetitive sequences in the genome. In the few cases where a RFLP pattern of low complexity was obtained, the polymorphic fragments could be mapped at the same position as their cognate amplified products. Our results are consistent with findings in other species (Williams et al. 1990) and indicate that RAPDs can be fruitfully used to mark genomic regions which, due to the presence of repetitive DNA sequences, are difficult to access by RFLP analysis.

The present state of the map

In Table 1 we have summarized the available cytogenetical data showing the correlation of the karyotype of *Beta vulgaris* chromosomes with linkage groups based on mutants, isoenzymes, RAPDs and RFLPs. In the table the numbering of chromosomes is based on the standard karyotypes of Bosemark and Bormotov (1971), as used by Romagosa et al. (1987) to identify their series of trisomics cytogenetically. The correspondence between the trisomics of Romagosa et al. (1986) and those of Butterfass (1964) is based on assigning a specific karyotype to the trisomics of Butterfass I, II, III, IV and VIII (Romagosa et al. 1986; recent personal communications of I. Romagosa and J.M. Lasa have confirmed these assignments). The genes reported in column 5 of Table 1 have been allocated

Table 1 Summary of available cytogenetical data showing the correlation of the karyotype of *Beta vulgaris* chromosomes with linkage groups, based on isoenzyme, RAPD or RFLP analyses

Numbering of *Beta vulgaris* chromosomes based on the standard karyotype of Bosemark and Bormotov (1971)	Trisomics of *Beta vulgaris* identified cytologically by Romagosa et al. (1987)	Correspondence[a] between the trisomics of Romagosa et al. (1986) and those of Butterfass (1964)	Genes allocated to chromosomes based on the trisomics of Butterfass (1964)	Genes linked to those allocated to chromosomes	Linkage groups corresponding to the trisomic series of Romagosa et al. (1987)		
					Barzen et al. (1992)	Uphoff and Wricke (1992)	Wagner et al. (1992)
1	Type 1	I	*Lap1*[b]	*AK1*[u]			
2	Type 2	–					
3	Type 3	II	*R*[c], *Got3*[b], *Icd1*[d]	*Y*[e], *B*[e], *C*[f], *Got3(2)*[g], *R*[h], *Est2*[i], *Fdp2*[i], *Icd1(2)*[n]	VII[o]	II[p]	I[q]
4	Type 4	III	*Mdh1*[d]	*Est5*[i], *Rr1*[j], *Mdh1*[i], *X*[k], *Z*[l], *Nb*[m], Gdh_2[u]			III[r]
5	Type 5	IV	*Aco1*[d]	*M*[i], *Est3*[i], *Aco1*[i], *Fas*[i]	III[s]		II[t]
6	Type 6	–					
7	Type 7	–					
8	Type 8	VIII					
9	Type 9	–					

[a] Romagosa et al. (1986); Nakamura et al. (1991)
[b] Oleo et al. (1993) as cited in Lange et al. (1993)
[c] Butterfass (1968)
[d] Lange et al. (1993)
[e] Keller (1936); Owen and Ryser (1942); Owen et al. (1940); Abegg (1936) as cited in Abe et al. (1993)
[f] Cited in Pillen et al. (1992; 1993)
[g] Abe and Tsuda (1987); Wagner et al. (1992); Abe et al. (1993)
[h] Pillen et al. (1992); Wagner et al. (1992); Abe et al. (1993)
[i] Wagner et al. (1992)
[j] Barzen et al. (1992)
[k] Pillen et al. (1993); Wagner et al. (1992)
[l] Van Geyt et al. (1990)
[m] Savitsky (1952; 1958)
[n] Smed et al. (1989); Wagner et al. (1992); Abe et al. (1993)
[o] *R* maps to this group
[p] *R* maps to this group
[q] *R*, *lcd1*, *Got3(2)*, *Est2*, *Fdp2* map to this group
[r] *Est5* maps to this group
[s] *M* maps to this group
[t] This group hosts an *Aco* locus most probably identical to the one mapped by Lange et al. (1993); *M* maps to this group
[u] Abe et al. (1993)

to the four Butterfass trisomics I, II, III and IV – which have an extra chromosome corresponding to the karyotype of chromosomes 1, 3, 4 and 5, respectively. The same genes allow the correlation of chromosomal karyotypes 3, 4 and 5 with available linkage groups based on isoenzymes, RAPDs and RFLPs. These three karyotypes correspond to the linkage groups I, III and II, respectively, of Wagner et al. (1992). Moreover, karyotypes 3 and 5 correspond to linkage groups VII and III, respectively, of Barzen et al. (1992), while karyotype 3 is most likely to be associated with linkage group II of Uphoff and Wricke (1992).

The linkage groups of Fig. 1 are numbered as follows: group 3 and 5 according to their chromosomal assignment; and the remaining chromosomes as described by Barzen et al. (1992). In Fig. 1, the loci revealed by PCR are shown in italics and the loci with alleles segregating with abnormal ratios are followed by an asterisk. The finding of loci with alleles having a distorted segregation ratio in sugar beet is not new (discussed in Wagner et al. 1992 and Pillen et al. 1993). In our map such loci seem to be located all along linkage group VI, at the end of linkage group 3 and at an intermediate map position of group VIII. A lower or higher than normal transmission of specific gamete types can be due to the action of self-incompatibility alleles: four SI loci have been described in sugar beet (Larsen et al. 1977). The existence of gametic or zygotic lethal alleles has been reported for this species and can be an additional source of segregation distortion (Pillen et al. 1993). Structurally abnormal chromosomes (discussed in Barzen et al. 1992) may, however, also induce the skewed segregation of genetic markers. The possibility that such chromosomes were present in our mapping population could not definitely be excluded. We have hypothesized (Barzen et al. 1992) that a translocation may have been present in one parent of the cross. Moreover, local disturbances of recombination were noted in several of our linkage groups, indicated by groups of markers which did not recombine. These were found on most chromosomes except 5 and VIII.

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