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Chromosomal assignment of the nine linkage groups of sugar beet (*Beta vulgaris* L.) using primary trisomics

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Abstract Twenty-four marker loci representing each of the nine linkage groups of sugar beet (*Beta vulgaris*) have been assigned to the nine primary trisomics of Butterfass (1964). Single-copy RFLP probes were hybridized with filter-bound DNA of the trisomics. The autoradiographs were scanned and analyzed by densitometric methods. Statistics on the integrated optical densities of the RFLP bands revealed a clear relationship of each linkage group to a distinct trisomic type. For the first time each of the linkage groups could unequivocally be assigned to one sugar beet chromosome. A standard nomenclature of the 9 chromosomes of sugar beet is suggested and discussed with respect to previous numbering systems.

Key words *Beta vulgaris* · Chromosomal assignment · RFLP · Primary trisomics

Introduction

The association of linkage groups and single genes or markers with their respective chromosomes is an elementary step in developing a genetic map of a given species. Strategies to assign linkage groups to chromosomes and the correlation of genetic and physical maps are dependent on either chromosome mutations or high-resolution cytogenetic approaches, including in situ hybridization. Aneuploid material as well as substitution, addition and nullisomic lines, translocations and deletions of chromosomes are often used to locate molecular markers in plants.

Sugar beet (*Beta vulgaris* L.) is a true diploid species with 18 chromosomes (x = 9). Neither monosomic nor nullisomic stocks are available because the loss of chromosomes is not tolerated. However, two sets of primary trisomics have been established (Butterfass 1964, 1968; Romagosa et al. 1986, 1987). The nine trisomic lines developed by Butterfass (1964; and for chromosome IX, unpublished) are characterized by a heterogeneous genetic background and have been identified through the morphology of the plants. Their designation was arbitrary with the exception of chromosome I which is the largest of the complement. The trisomic set developed by Romagosa et al. (1986, 1987) has been characterized by its karyotype. However, the similarity in size and morphology of sugar beet chromosomes makes their unequivocal identification difficult (de Jong and De Bock 1978; de Jong et al. 1985; Löptien 1985; Nakamura et al. 1991). Recently, fluorescent in situ hybridization (FISH) has been used for the physical mapping of ribosomal genes, microsatellites and transposable DNA sequences on *Beta* chromosomes (Schmidt et al. 1994; Schmidt et al. 1995; Schmidt and Heslop–Harrison 1996; Schondelmaier et al. 1997).

Several linkage maps of sugar beet are available which are based on restriction fragment length polymorphisms (RFLPs), random amplified polymorphic DNAs (RAPDs) and on amplified fragment length polymorphisms (AFLPs) (Pillen et al. 1992, 1993; Barzen et al. 1992, 1995; Uphoff and Wricke 1995; Schondelmaier et al. 1995, 1996; Halldén et al. 1996; Barnes et al. 1996; Schumacher et al. 1997). However, the correlation between linkage groups and the 9 chromosomes of sugar beet is largely unknown. Primary trisomics are a common tool for assigning single markers or linkage groups to specific chromosomes (Young et al. 1987; Ellis and Cleary 1988; Lange et al. 1993; Oleo et al. 1993; Frary et al. 1996). The assignment of markers from linkage groups to chromosomes

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using primary trisomics can be accomplished in different ways. Distorted segregation or triallelic variation at a given marker locus serves to identify crucial crosses made with whole sets of trisomics with diploid plants. The chromosomal localization of a marker locus can be determined by fluorescent in situ hybridization (FISH) as has been recently described by the mapping of the 5S rRNA locus in sugar beet (Schondelmaier et al. 1997). However, only tandemly repeated repetitive sequences can be mapped in this way. Alternatively, dosage shifts can be observed after densitometrical analysis of Southern autoradiograms or isozyme gels (Young et al. 1987; Lange et al. 1993; Oleo et al. 1993). The technique of dosage analysis takes advantage of the fact that the strength of the hybridization signal produced by a molecular marker is proportional to the number of copies of that DNA marker in the genome. Hence, the signal from a marker located on the chromosome of which the trisomic has extra copies will be stronger in trisomic individuals than in diploids or non-crucial trisomics.

Here, we describe the assignment of RFLP markers previously mapped on the sugar beet genome to the 9 chromosomes of the trisomic series of Butterfass (1964) using Southern hybridization followed by densitometrical analysis of the autoradiograms.

Materials and methods

Plant material

A complete series of sugar beet primary trisomics ($2n = 18 + 1$) was grown under greenhouse conditions. The trisomic lines IV, VI and VIII were kindly provided by W. Lange, (Wageningen, The Netherlands) and others were obtained from T. Butterfass (Frankfurt, Germany). The original numbering of trisomic lines with Roman figures was used (Butterfass 1964). Trisomic plants were selected among the offspring of the original trisomics by counting the number of chromosomes after preselection on the basis of plant morphology, with the exception of type IX which did not show unique morphological features. At least 2 plants of each type were used for the molecular analysis (Table 1).

DNA isolation, probes, labeling and radioactive Southern hybridization

DNA was isolated from fresh or freeze-dried sugar beet leaves according to a standard CTAB-protocol (Saghai-Maroof et al. 1984). The DNA from trisomic plants was adjusted to a concentration of 0.25 µg/µl by comparing the amounts with known quantities of λ-marker (Pharmacia) and digested to completion with *PstI*. Between 5 and 10 µg of digested DNA per lane were separated in 1.0% agarose gels overnight and blotted onto nylon membrane using $20 \times$ SSC buffer. Twenty-four clones from our current linkage map (Schondelmaier et al. 1995; Schumacher et al. 1997) were selected. Plasmid inserts were radioactively labeled according to Pillen et al. (1992). Only true single-copy clones were chosen showing clear hybridization patterns with the mapping population and the trisomics. The hybridization of blots, washing steps and signal detection were performed according to Pillen et al. (1992).

Densitometry and statistical analysis

Densitometry was performed on 24 Southern autoradiographs by scanning them with a scanner (Pharmacia Ultroscan XL) connected with a SUN SPARC 10 workstation. Tiff images were analyzed with RFLPScan 3.0 software (Scanalytics) running on a Pentium computer. The obtained integrated optical densities (Int. OD, i) of the RFLP bands were z-transformed (z_i), which enables comparisons between Southern experiments of different probes to the same blot:

$$z_i = \frac{i - \mu_i}{s_i}$$

where μ_i is the mean of all Int. OD from a single hybridization experiment with 1 Southern blot, and s_i is the Standard deviation of all Int. OD from a single hybridization experiment with 1 Southern blot.

Table 1 Number of statistically analyzed experiments obtained by hybridization of single-copy probes of our RFLP map of sugar beet (Schondelmaier et al. 1995) to Southern blots of trisomics (designation according to Butterfass 1964). Each value of integrated optical density obtained after densitometry of trisomic DNA hybridized with a single-copy probe represents one datapoint. Bold letters indicate the number of datapoints on which the identification of the linkage groups to the proper trisomic type is based. The number of experiments was reduced for those probes of which the chromosomal assignment was already known. On average, 66 (596/9) datapoints per linkage group were investigated

	Linkage group	I	II	III	IV	V	VI	VII	VIII	IX	Total
	Number of probes	2	3	1	3	4	3	1	3	4	24
Trisomic type	Number of plants										
B-I	3	3	6	3	6	**13**	9	4	9	13	66
B-II	2	**2**	4	2	4	7	5	2	5	8	39
B-III	2	2	4	**2**	4	8	6	2	6	8	42
B-IV	3	1	**4**	1	6	10	9	3	7	10	51
B-V	4	4	8	4	8	17	12	5	**12**	17	87
B-VI	5	0	5	0	9	15	14	**5**	9	14	71
B-VII	7	4	11	4	14	25	**21**	7	18	25	129
B-VIII	4	0	4	0	8	12	12	4	8	**12**	60
B-IX	3	1	4	1	**6**	10	9	3	7	10	51
Total	33	17	50	17	65	117	97	35	81	117	596

A further normalization (z_i') enabled the final comparisons between different Southern experiments with different probes to all blots according to the formula

$$z_i' = z_i - \mu_z,$$

where μ_z is the mean of all residual z_i belonging to a distinct trisomic of 1 blot (i.e. mean_{row}). The normalized data (z_i') were used for simple statistics (ANOVA).

All data obtained with probes from one linkage group hybridized with DNA of one trisomic type were handled as repeats. Standard multiple mean tests (LSD, least significant difference) with a threshold of $\alpha = 5\%$ were achieved by the GLM procedure of SAS software (SAS Institute 1989).

Results

Because trisomic lines of each of the 9 sugar beet chromosomes are available (Butterfass 1964), it is possible to assign sugar beet genomic clones to specific chromosomes by observing increased hybridization of a given probe to DNA of one of the trisomic lines. In theory, the dosage shift is from 100% in diploids to 150% in a crucial trisomic. First, we used a simplified RFLP procedure which had been successfully applied with the inbreeding crop tomato (Young et al. 1987). Up to 5 probes were pooled for a single hybridization experiment. It was expected that the hybridization of *PstI*-digested DNA from trisomic plants would result in monomorphic banding patterns. The probe combinations were assembled in a way that the expected banding pattern should unequivocally be identified. However, after 3 such hybridizations complex polymorphic banding patterns became visible due to the outbreeding character of sugar beet. It was not possible to track single bands of the same molecular weight. Therefore, it was necessary to hybridize each probe separately resulting in 24 readily scorable autoradiograms, which were further analyzed after scanning (Fig. 1).

We did not rely on the comparison of band intensities directly after scanning because of the following reasons: (1) the amount of DNA loaded into each lane may be variable and (2) the signals between different hybridization experiments may vary in strength. These features were overcome by using a statistical approach on the densitometrically obtained results. Integrated optical densities of the RFLP bands which were provided after image analysis by the RFLPscan software were z-transformed and normalized to allow comparisons between hybridization experiments of the probes to the blots. These transformed and normalized values, which were normally distributed, were investigated with ANOVA statistics and LSD tests (Table 2). As a result, significantly different means were calculated for trisomics I through VIII (Table 2). For trisomic IX the means from linkage groups IV and VII were not significantly different. On the basis of the statistical analysis all nine linkage groups could unequivocally be assigned to 1 of the sugar beet chromosomes. The

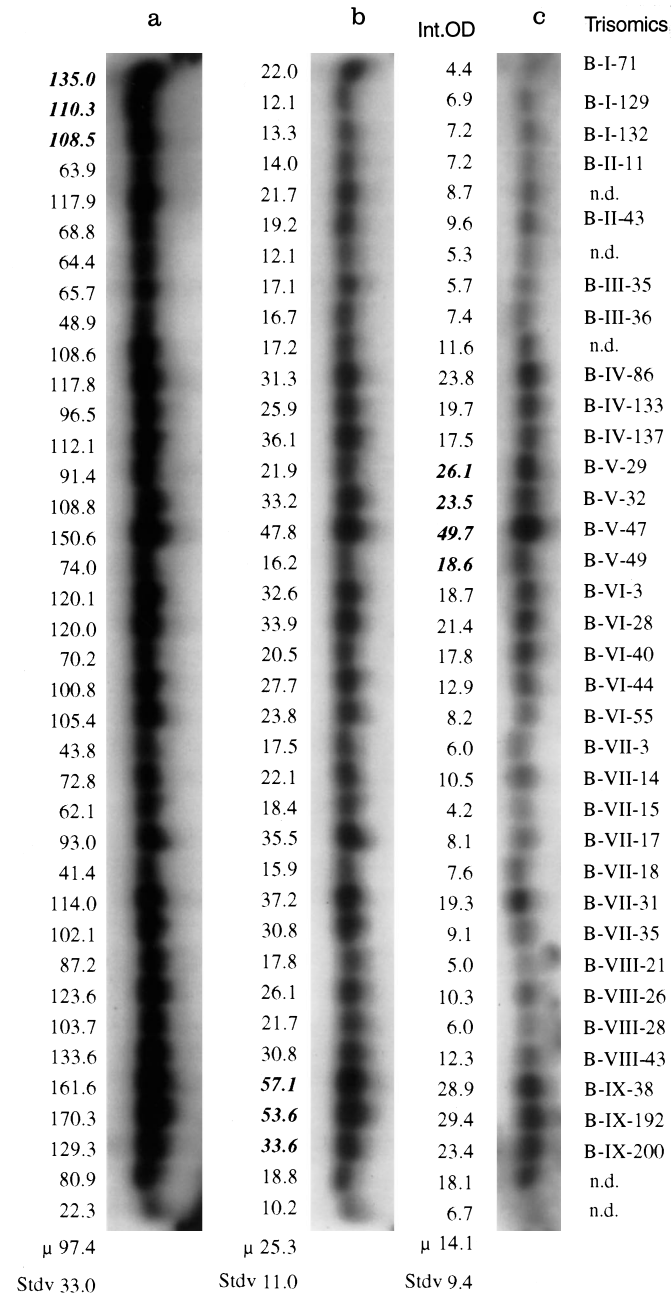

Fig. 1a–c Autoradiographs of three single-copy probes hybridized to DNA of trisomics. Individual trisomic plants are numbered, e.g. B-I-71 is plant no. 71 from the trisomic type I. **a** Strong signals after hybridization with probe *pKP543* from linkage group V. The critical trisomic is type I. Exposure time: 14 days. **b** Medium signals after hybridization with probe *pKP511* from linkage group IV. The critical trisomic is type IX. Exposure time: 10 days. **c** Weak signals after hybridization with probe *pKoeln141* from linkage group VIII. The critical trisomic is type V. Exposure time: 7 days. Differences in hybridization intensity between trisomics were not detectable by the naked eye. Therefore, autoradiographs were scanned, and the integrated optical densities (Int. OD) were calculated by image analysis as described in the Materials and methods

Table 2 Results of the multiple mean test obtained by statistical analysis of the hybridization signals of all linkage group/trisomic combinations. The linkage groups (rows) are in Roman numbers (Schondelmaier et al. 1995), the trisomics (columns) are designated with B-I through B-IX according to Butterfass (1964). Different letters indicate significantly different means. The mean value indicated by an 'A' is significantly different from the other means of the same trisomic, thus demonstrating a stronger hybridization signal of probes from a given linkage group to one trisomic. In this way the correlations between linkage groups and chromosomes could be revealed, e.g. linkage group V corresponds to Butterfass trisomic type I (chromosome I) (*n.d.* not determined)

Linkage groups	Trisomics									
	B-I		B-II		B-III		B-IV		B-V	
	Grouping	Mean	Grouping	Mean	Grouping	Mean	Grouping	Mean	Grouping	Mean
I	B	0.09	A	1.35	D	− 0.76	B	0.00	C B	− 0.26
II	B	− 0.12	B	0.25	C B	0.00	A	1.96	B	− 0.05
III	B	− 0.40	C B	− 0.18	A	0.74	C	− 0.77	B	0.00
IV	B	− 0.03	C B	0.20	B	0.16	B	− 0.11	B	− 0.05
V	A	1.25	C B	− 0.15	C B	− 0.10	B	− 0.12	C B	− 0.26
VI	B	− 0.49	C	− 0.24	C	− 0.32	C B	− 0.26	C	− 0.63
VII	B	− 0.20	C B	− 0.03	C B	− 0.02	B	0.06	B	0.05
VIII	B	− 0.36	C B	0.05	B	0.20	C B	− 0.30	A	1.32
IX	B	− 0.46	C	− 0.26	B	0.11	B	− 0.10	B	− 0.10

Table 2 Continued

Linkage groups	Trisomics									
	B-VI		B-VII		B-VIII		B-IX			
	Grouping	Mean	Grouping	Mean	Grouping	Mean	Grouping	Mean		
I		n.d.	B	0.10		n.d.	B C	− 0.21		
II	C B	− 0.13	B	− 0.10	D	− 0.92	C	− 0.87		
III		n.d.	B	0.05		n.d.	B C	− 0.12		
IV	C B	0.10	C B D	− 0.23	C B	− 0.24	A	0.99		
V	C B	− 0.10	D	− 0.46	B	0.12	B	0.12		
VI	C B	− 0.13	A	1.21	C B	− 0.14	B C	− 0.15		
VII	A	0.98	B	0.06	E	− 1.65	B A	0.19		
VIII	B	0.16	C D	− 0.44	C D	− 0.44	B C	− 0.11		
IX	C	− 0.24	C B	− 0.11	A	1.32	B C	− 0.18		

T-test statistics: least significant differences (LSD), $\alpha = 5\%$

correlation between linkage groups and chromosomes together with a literature review of previously mapped marker genes is presented in Table 3.

Triallelic variation revealed by a single-copy RFLP probe is another means of assigning the marker locus to a trisomic chromosome. In our experiments, only 1 probe was found to identify a triallelic heterozygote with one of the trisomics. The probe *pKoeln106* from linkage group VII (Schondelmaier et al. 1995) yielded three bands with the B-IV trisomic line (Fig. 2), demonstrating that this linkage group is associated with chromosome VI of sugar beet. This finding fits into the results from the statistical analysis (Table 2).

Discussion

In principle, three different strategies are available for assigning linkage groups to chromosomes using single-copy RFLP markers. First, the presence of a third allele within trisomic stocks from a true diploid species indicates the location of the corresponding RFLP locus. Second, the chromosomal location of the locus in question can be deduced from the distorted segregation ratios in the offspring of trisomics. The presence of an additional chromosome in a trisomic modifies the expected Mendelian segregation ratios for genes on that chromosome (Lange et al. 1993; Oleo et al. 1993). Here, the application of this method is hampered by the fact that in experimental populations of sugar beet several regions in the genome show severely distorted segregation (Oleo et al. 1993; Pillen et al. 1993; Schondelmaier et al. 1995, and unpublished results). Therefore, this approach may result in a false grouping of markers, especially if the crosses were not accomplished with the whole trisomic stocks but only with a subset (Butterfass 1968; Oleo et al. 1993).

The third strategy makes use of single-copy RFLP markers and densitometric analysis of bands obtained after Southern hybridization of trisomics. Compared to mapping studies based on segregation distortion only

Table 3 A synopsis of all genes previously used in mapping studies of sugar beet and their location on the nine chromosomes according to the trisomic set of Butterfass (1964; modified and expanded from Barzen et al. 1995). The probes used in this study are in bold letters (Schondelmaier et al. 1995)

Trisomics of Butterfass (1964)	Trisomics of Romagosa et al. (1986)[a]	Marker loci mapped to Butterfass trisomics	Genes located on linkage groups and chromosomes	Linkage groups corresponding to the trisomic series of Butterfass (1964)		
				Barzen et al. (1995)	Pillen et al. (1993)	Uphoff and Wricke (1995)
I	Triplo 1	*Lap1*[b], 18S rRNA[x], ***pKP975***, ***pKP543***,	*Ak1*[u], *Let5b*[k] *PsbS*[s], *PetE*[s]	VI	V	
II	Triplo 3	*R*[c], *Got3*[b], *Icd1*[d], ***pKoeln42***, ***pKP814***	*Y*[e], *B*[e,f,u], *C*[g], *Est2*[i], *Fdp2*[i], *Got3(2)*[i,u], *Letla*[k], *Nema*[t]	3	I	I[n]
III	*Triplo* 4	*Mdh1*[d], ***pKP1238***	*Est5*[i], *X*[i,j,u], *Rr1*[b], *Gdh2*[u], *Skdh2*[q]	IV	III	III[l]
IV	Triplo 5	*Aco1*[d], 5SrRNA[y], ***pKoeln7***, ***pKoeln143***, ***pKP876***	*Est3*[i], *Z*[i,o], *M*[h,i], *Fas*[i], *Nb*[m], *Sc*[m], *PsbO*[s], *cTRF*[s], *PsaL*[s], *PsbR*[s], *Pgm1*[p], *Mdh3*[p]	5	II	
V	Triplo 6?	***pKP849***, ***pKoeln141***, ***pKP782***	*Est1*[i], *Let8*[k]	IX	VIII	
VI	Triplo 7	***pKP1184***, ***pKoeln106***	*PetH*[s], *PsbP*[s]	VIII	VII	
VII	Missing?	***pKoeln79***, ***pKoeln75***, ***pKP1227***	*Dia1*[i], *PsaE*[s]	VII	VI	VI[v]
VIII	Triplo 8	***pRH1246***, ***pkP847***, ***pKP574***, ***pKoeln62***	*Acp1*[i], *PsbQ*[s]	II	IX	IX[w]
IX	Triplo 2? and 9?	***pKP942***, ***pKP501***, ***pKP511***	*Hs1*[r], *Hs2*[r], *PsaH*[s], *PetC*[s]	I	IV	

[a] Correspondence between the trisomics of Butterfass (1964) and those of Romagosa et al. (1986), Nakamura et al. (1991) and Schondelmaier, unpublished results
[b] Oleo et al. (1993)
[c] Butterfass (1968)
[d] Lange et al. (1993)
[e] Keller (1936), Owen et al. (1940), Owen and Ryser (1942)
[f] Bolting behavior *B*; Boudry et al. (1994)
[g] Root color, *Y*; curly top resistance, *C*; cited from Wagner et al. (1992)
[h] Resistance to rhizomania, *Rr1*; monogerm character, *M*; Barzen et al. (1995)
[i] Stem fasciation, *Fas*; restoration of male fertility, *Z* and *X*, and more isozyme loci (Wagner et al. 1992)
[j] Pillen et al. (1993)
[k] Severely distorted segregation, *Let1a*, *Let5b*, *Let8* (Pillen et al. 1993; Schondelmaier et al. 1995)
[l] *Mdh1* maps to LG III; Wagner et al. (1992)
[m] Late bolting, *Nb*, sugar content, *Sc* (Savitsky 1952, 1958)
[n] *R* maps to chromosome II (Butterfass 1968)
[o] *Z* might be linked with *M* (Roundy and Theurer 1974)
[p] *Pgm1* is linked with *Mdh3*, and *Pgm1* might be linked with *M* (Abe et al. 1993)
[q] *Skdh2* might be linked with a restorer gene, perhaps *X* (Abe et al. 1993)
[r] Resistance against *H. schachtii*, $Hs1^{pro-1}$, $Hs2^{pro-7}$ (Heller et al. 1996)
[s] Chloroplast protein genes *PsbS*, *PetE*, *PsbO*, *cTRF*, *PsaL*, *PsbR*, *PetH*, *PsbP*, *PsaE*, *PsbQ*, *PsaH*, *PetC* (Pillen et al. 1996)
[t] Nematode resistance gene, *Nema* (Uphoff and Wricke 1995)
[u] Abe et al. (1993)
[v] *Dia1* maps to linkage group VI (Pillen et al. 1993)
[w] *Acp1* maps to linkage group IX (Pillen et al. 1993)
[x] Schondelmaier et al., in preparation
[y] Schondelmaier et al. (1997)

a few trisomic plants are needed, thus reducing the time-consuming cytological control of trisomic offspring. The multiple probing approach as described by Young et al. (1987) turned out not to be practical in our study because it yielded complex polymorphic banding patterns. The presence of multiple alleles is due to the outbreeding character of sugar beet. Therefore, we had to hybridize 1 probe at a time to 1 blot containing digested DNA. For future studies, dot blot hybridization with DNA from the trisomic set is suggested so as to avoid the laborious production of Southern blots.

Before this study was started, the correlation between linkage groups and chromosomes of sugar beet was largely unknown with the following exceptions (for a review see Barzen et al. 1995). The isozymes loci *Got3*,

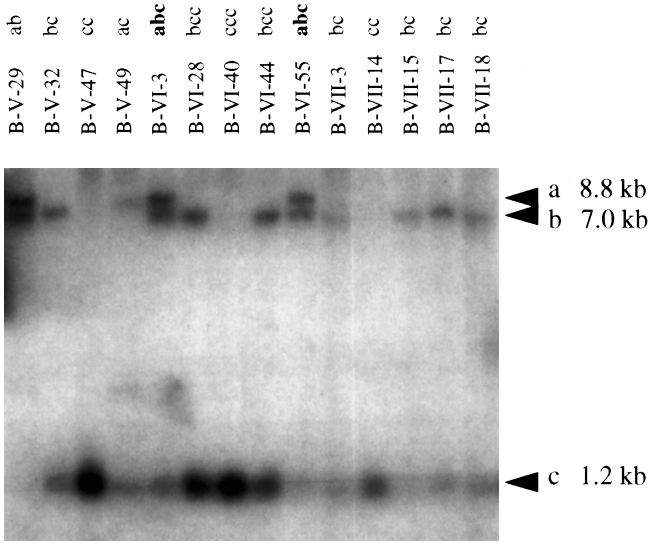

Fig. 2 Detection of a triallelic heterozygote after Southern hybridization with the probe *pKoeln106* from linkage group VII. Three bands (*a*, *b* and *c*) were visible only with DNA of trisomic plants 03 and 55 (trisomic type VI), indicating that LG VII corresponds to chromosome VI

Icd1 from linkage group I (Oleo et al. 1993; Lange et al. 1993) were assigned to the trisomic chromosome in the Butterfass type II (B-II). The isozyme loci *Mdh1* and *Aco1* from linkage groups III and II were found to be located on chromosomes III and IV, respectively (Lange et al. 1993). The gene for red hypocotyl color (*R*), which had been included on linkage group I (Pillen et al. 1992), had previously been assigned to chromosome B-II (Butterfass 1968). We adopted this mapping information to our studies in a way that the number of investigated clones was reduced for these linkage groups (Table 1). Our results were in agreement with published assignments, indicating the high reliability of all methods used (Table 3).

Using 24 single-copy RFLP markers which had been mapped previously to our sugar beet linkage map (Schondelmaier et al. 1995; Schumacher et al. 1997) in combination with the densitometric analysis of the bands obtained, we have assigned all linkage groups to the 9 chromosomes of sugar beet. We found that densitometry is an essential step in dosage studies, as shifts were hardly perceptible with the naked eye (cf. Fig. 1). The application of simple statistics upon the data obtained from image analysis revealed a clear assignment of the single-copy probes to distinct chromosomes. For the first time a link was established for this species between the genetic linkage map and all the physical carriers of the markers. We included all of the mapping information available from former studies to establish a comprehensive genetic map of sugar beet. We also extended previously published relationships between a linkage group and a sugar beet chromosome presented by Barzen et al. (1995) to all nine possible relations. In contrast to Barzen et al. (1995), who used the trisomic designation of Romagosa et al. (1987) for two linkage groups of their map, we adopted the designation of Butterfass (1964) for our map. The correlation of the karyotype of *B. vulgaris* chromosomes with all published linkage groups and linkage relations based on mutants, isoenzymes and molecular markers is shown in Table 3. Only two recently published sugar beet maps from private breeding companies based on RFLPs (Halldén et al. 1996) and RFLPs/AFLPs (Bartens et al. 1996) could not be included due to the lack of any morphological marker in these maps. This obstacle significantly reduces the value of these maps for future studies.

Various numbering systems have been proposed for sugar beet chromosomes, based on trisomic lines (Butterfass 1964; Kaltsikes and Evans 1967; Romagosa et al. 1987), the grouping of similar-sized chromosomes (Cistue et al. 1985) and pachytene banding patterns (de Jong and Stam 1985). Despite extensive karyotype analyses all of the methods failed to unequivocally distinguish between all nine chromosomes (Nakamura et al. 1991). Genetic maps have been published with arbitrary chromosome designations (Pillen et al. 1993; Barzen et al. 1992; Schondelmaier et al. 1995; Uphoff and Wricke 1995; Halldén et al. 1996; Barnes et al. 1996). Barzen et al. (1995) proposed the integration of their map with the trisomics of Romagosa et al. (1987). We have also included several trisomic types of the Romagosa complement in or studies (data not shown). However, only seven out of nine possible primary trisomics were available because triplo 6 is lethal (Romagosa et al. 1987) and triplo 5 did not germinate in our hands. Therefore, the numbering systems of Butterfass (1964) was used, who produced the first complete trisomic series which is still readily available. We suggest that this standard nomenclature of the nine chromosomes be adopted for future genetic studies on sugar beet.

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