Theor Appl Genet (1992) 84:129–135

THEORETICAL AND APPLIED GENETICS

A linkage map of sugar beet (*Beta vulgaris* L.)

K. Pillen [1], G. Steinrücken [2], G. Wricke [3], R.G. Herrmann [1], and C. Jung [1,*]

[1] Botanical Institute of the Ludwig-Maximilians University Munich, Menzinger Strasse 67, W-8000 Munich 19, FRG
[2] A. Dieckmann-Heimburg, Saatzucht Sülbeck, Kirchhorsterstrasse 2, W-3065 Nienstädt, FRG
[3] Institute of Applied Genetics, University of Hanover, Herrenhäuser Str. 2, W-3000 Hanover 21, FRG

Received September 23, 1991; Accepted October 17, 1991
Communicated by G. Wenzel

Summary. We have established a first linkage map for beets based on RFLP, isozyme and morphological markers. The population studied consisted of 96 F_2 individuals derived from an intraspecific cross. As was expected for outbreeding species, a relatively high degree of polymorphism was found within sugar beet; 47% of the DNA markers were polymorphic for the chosen population. The map consists of 115 independent chromosomal loci designated by 108 genomic DNA probes, 6 isozyme and one morphological marker. The loci cover 789 c*M* with an average spacing of 6.9 c*M*. They are dispersed over nine linkage groups corresponding to the haploid chromosome number of *Beta* species. Eighteen markers (15.4%) showed distorted segregation which, in most instances, can be explained by gametic selection of linked lethal loci. The application of the linkage map in sugar beet breeding is discussed.

Key words: Sugar beet – *Beta vulgaris* – RFLP markers – Linkage map – Distorted segregation

Introduction

Relative to the economical importance of sugar beet (*Beta vulgaris* L.), the amount of genetic data available on this species is relatively limited. The cultured beet belongs to the genus *Beta* (Chenopodiaceae) which includes fodder beet, swiss chard, red table beet and numerous wild beet species that are useful as gene resources (Van Geyt et al. 1990a). It is a diploid species with 18 chromosomes (n = x = 9) and a nuclear DNA content per haploid genome of 1.25 pg (Bennet and Smith 1976), resulting in a total genome length of approximately 1.15×10^9 base pairs. Beets are characterized by a biannual behaviour and self-sterility resulting from the interaction of four gametophytic, complementary S-loci (Larsen 1977). Diploid and triploid hybrid varieties are available; the triploids result from crosses between male-sterile, diploid lines and tetraploid pollinators. Yield, sugar content and other quality components are important breeding characters together with bolting resistance, monocarpy and disease resistances. Since *B. vulgaris* displays polycarpic seedballs, single-germ breeding lines have been selected (Barocka 1962).

Little data on linkage relationships in *Beta* have been published so far. An overview of morphological and isozyme markers has been given by Van Geyt et al. (1990b) and Wagner and Wricke (1991). Alien addition lines carrying monosomic chromosomes from the wild species *B. procumbens* were used for the chromosomal localization of isozyme markers. With this strategy, the only larger linkage group, Y-R-B (Keller 1936; Owen and Ryser 1942), including the isozyme genes *Got* and *Icd-2* (Smed et al. 1989), *Est-2, Fdp-2* and the gene for red hypocotyl colour (Wagner and Wricke 1991), was localized on chromosome I (Van Geyt et al. 1988). However, the isozyme pattern between both species is not always consistent, and the arrangement of the genes on the homoeologous chromosomes remains to be checked.

Obviously, RFLP markers have a wider potential in the genetic mapping of complex genomes than isozyme markers because of their almost unlimited number and codominant expression. Exhaustive linkage maps based on RFLP markers have been presented for several economically important species such as maize (Coe et al. 1988), tomato (Tanksley 1988), lettuce (Landry et al. 1987), potato (Gebhardt et al. 1989), *Brassica oleracea* (Slocum et al. 1990), rice (McCouch et al. 1988) and bar-

* Correspondence to: C. Jung

ley (Graner et al. 1991). The strategies which had been chosen for mapping genomes of higher plants depend on the degree of genetic polymorphism within the species and the complexity of the genome. (1) Generally, segregating populations derived from intraspecific or interspecific crosses are chosen. In case of a low degree of intraspecific polymorphism the latter material has proven useful (Bonierbale et al. 1988). (2) Recombinant inbreds (Burr and Burr 1991) and double-haploids (Graner et al. 1990, 1991) possess the advantage that the plant material can be maintained indefinitely. (3) Alien addition lines, nullisomic lines and translocations involving chromatin from different species has proven helpful in mapping chromosomes in allopolyploid species like wheat (Chinoy et al. 1991) and in selecting markers for defined regions of a genome that had not been mapped in any way before (Jung et al. 1990).

Molecular work with sugar beet has so far been restricted to mitochondrial DNA (mtDNA) (Ecke and Michaelis 1990; Weihe et al. 1991), plastid DNA (Fritzsche et al. 1987) and to repeated nuclear DNA elements (Schmidt et al. 1991). In a strategy based on alien addition and translocation lines, DNA probes have been selected that can be used as markers for nematode resistance in sugar beet. Attempts have been made to clone this gene, which is derived from wild species of the Procumbentes section, by the techniques of reverse genetics (Jung et al. 1990; Jung et al. in press). In order to select markers for other genes of interest as well, an integrated map of the sugar beet genome including RFLP, isozyme and morphological markers has been established. Its use in breeding programmes is discussed.

Materials and methods

Plant material

A male-sterile breeding line was crossed with five restorer populations (A. Dieckmann-Heimburg, Saatzucht Sülbeck). F_2 populations were created by selfing individual F_1 plants. One of these populations (S227), consisting of 96 diploid plants, was used for segregation analysis.

Isozyme analysis, morphological characters and DNA extraction

Seven isozyme families were analyzed (*Acp-1, Est-1, Est-3, Est-5, Dia-1, Got-2, Icd-1*) following the protocol given by Wagner and Wricke (1991). Hypocotyl colour (R) and fertility (X) were also determined. Total DNA was extracted from developing, green leaves following the protocol of Dellaporta et al. (1983).

Plasmid probes and identification of single-copy probes by dot-blot hybridization

Genomic sugar beet DNA (10 µg) was completely digested with *Pst*I (Boehringer, Mannheim) and separated on 1% agarose gels. DNA in the range from 1 to 2 kbp was electroeluted in 1 × TA onto a NA45 membrane (Schleicher and Schüll, Dassel) with 10 V/cm. DNA was released from the membrane and washed under the conditions specified by the supplier. The DNA was then cloned into dephosphorylated plasmid vector "Bluescribe M13$^+$" (Stratagene). *E. coli* DH5α served as a host. *Eco*RI- and *Bam*HI-based libraries (Jung et al. 1990) were used as controls. Two photosystem-II cDNA probes from spinach coding for the 20-kDa protein (c20kD) and the 22-kDa protein (c22kD) were included in the mapping experiments (Herrmann et al. 1991).

Repetitive genomic clones were identified by dot-blot hybridization. Two micrograms plasmid DNA was denatured with 0.4 *M* NaOH and 1 *M* NaCl and blotted onto a Biodyne B membrane (Pall Filtrationstechnik, Dreieich) using a vacuum dot-blot apparatus (Schleicher und Schüll, Dassel). The blots were then hybridized with ^{32}P-labeled nuclear DNA from sugar beet (see below).

Gel electrophoresis, probe labeling and Southern hybridization

Genomic DNA was restricted with the enzymes *Eco*RI, *Eco*RV, *Hin*dIII or *Xba*I and separated on 0.75% agarose slab gels overnight. The DNA was transferred to a Biodyne B membrane using the modified SSC technique described by the manufacturer.

Plasmid inserts were separated in 1.3% low-gelling agarose and labeled to specific activities of approximately 1×10^9 cpm/µg by random priming (Feinberg and Vogelstein 1983) in the presence of α ^{32}P-dCTP (3000 Ci/mMol, Amersham) using "random priming" kits (Boehringer, Mannheim) and 25 ng DNA per reaction.

The hybridization was carried out in 5 × Denhardt's solution, 5 × SSPE and 0.2% SDS with herring sperm DNA (200 µg/ml). Post-hybridization wash was 0.5 × SSC, 0.2% SDS at 65 °C followed by 0.1 × SSC, 0.2% SDS. The membranes were rehybridized after stripping with 0.2 *N* NaOH at room temperature.

Linkage analysis

Autoradiograms were scored after 5 days of exposure. Expected segregation ratios (1:2:1 for DNA and isozyme markers, 3:1 for morphological markers and *Est-5*) were tested by χ^2 analysis. In the cases of highly significant deviations ($\alpha = 0.01$) from the expected ratio for codominant segregation, a test for gametic selection (unequal frequencies of both homozygous classes) was made. The recombination value (c) between marker and selective lethal gene locus was computed with the maximum likelihood method using the formula from Wagner et al. (1992) with $c = A/(A+B)$. "A" denotes the frequency of the minor homozygous class, whereas "B" indicates the superior homozygous class.

Linkage analysis for all markers was performed using the computer programme MAPMAKER (Lander et al. 1987). The linkage groups were formed with linkage criteria of LOD = 4.0 and 0.23 recombination units. Multipoint linkage map distances were calculated using the Kosambi transformation (Kosambi 1944).

Results

Probe selection

Three randomly cloned plasmid libraries have already been established from genomic *Beta*-DNA digested with the CXG-methylation-sensitive enzyme *Pst*I and the non-sensitive enzymes *Bam*HI and *Eco*RI (Jung et al. 1990). After hybridization with filter-bound sugar beet

DNA, three classes of probes were identified, i.e. single- or low-copy probes detecting 1–6 bands, high-copy probes with a polymorphic banding pattern (up to 30 bands) and probes hybridizing with tandemly repeated DNA elements that display one strong signal. Generally, only probes from the first category are suitable for RFLP mapping. Therefore, the libraries were screened prior to genomic hybridization using the dot-blot technique. Due to the strength of their hybridization signals, high-copy probes could be easily identified; conversely, plasmids with low-copy inserts gave only weak signals (Fig. 1). There were clear differences between the plasmid libraries with respect to single-copy inserts (Fig. 2). The highest frequency was found in the *Pst*I library (93%), the lowest in the *Bam*HI library (11.5%). The *Eco*RI-based library gave an intermediate pattern (37%).

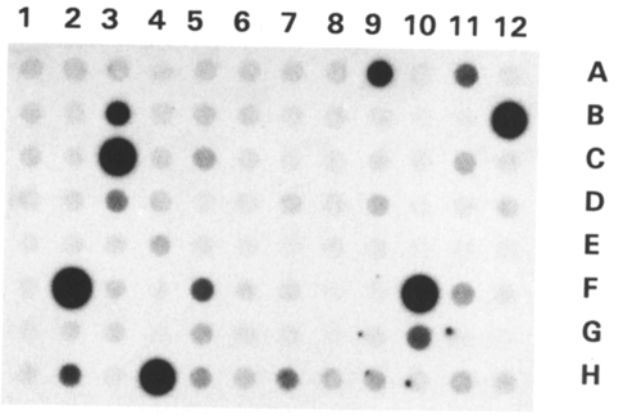

Fig. 1. Dot-blot screening of 96 plasmid probes with ^{32}P-labeled nuclear DNA of sugar beet. Plasmids containing single-copy inserts reveal weak signals (e.g. *A1*), whereas repeated sequences give strong signals (e.g. *B12*). The latter ones were excluded from RFLP analysis. Filter exposure: 8 days

Linkage mapping

Sugar beet DNA was restricted with the four enzymes *Eco*RI, *Eco*RV, *Hin*dIII and *Xba*I and separated on 0.75% agarose gels. These enzymes proved to be most informative with respect to RFLPs, however differences were also found between them (Fig. 3). The most polymorphic fragments were detected by *Eco*RV (34%); *Hin*dIII proved to be less informative (19%). The average fragment size ranged between 6.6 kbp (*Hin*dIII) and 11.3 kbp (*Eco*RV).

Among the five segregating sugar beet populations, S227 displayed the highest frequency of RFLPs: of 204 *Pst*I probes tested, 47% were informative for this population. Consequently, the mapping experiments were restricted to this S227 population (Fig. 4). A total of 108 RFLP markers were mapped together with 7 isozyme (*Acp-1, Est-1, Est-3, Est-5, Dia-1, Got-2, Icd-1*) and 2 morphological markers (red hypocotyl R, restorer X; Fig. 5). Of the DNA markers, 58% displayed 2 bands; the highest number of bands (8) was detected by probe pKP747. Among the 117 markers employed in the mapping analysis, 18 (15.4%) showed distorted segregation; 17 of these may be explained by gametic selection. The genetic distances to hypothetical lethal loci range between 0.0 and 0.29 recombination units. The possible locations of 5 lethal genes are integrated into the map (Fig. 5).

Altogether the markers are dispersed over nine linkage groups corresponding to the haploid chromosome number of *Beta* species. The map comprises 789 total map units with an average distance of 6.9 cM (estimated total map units divided by the number of markers mapped). Fourteen loci, clustered in part, are located on group IV, which represents the shortest group (45.7 cM). In contrast, group VII comprises 140.9 cM with 16 markers. Under the conditions chosen, 2 markers (X and *Dia1*) could not be localized on any linkage group.

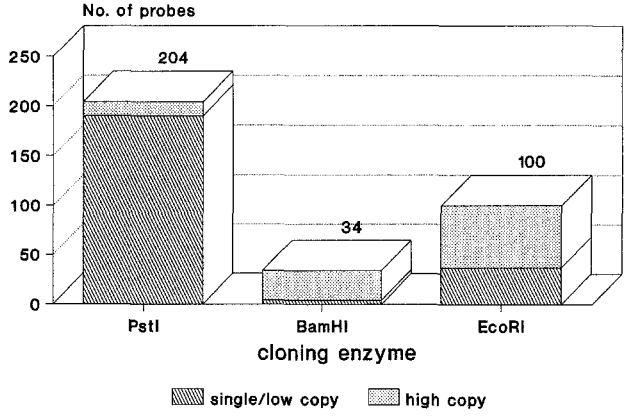

Fig. 2. The frequency of single-, low-copy and high-copy probes in three different, randomly cloned sugar beet plasmid libraries

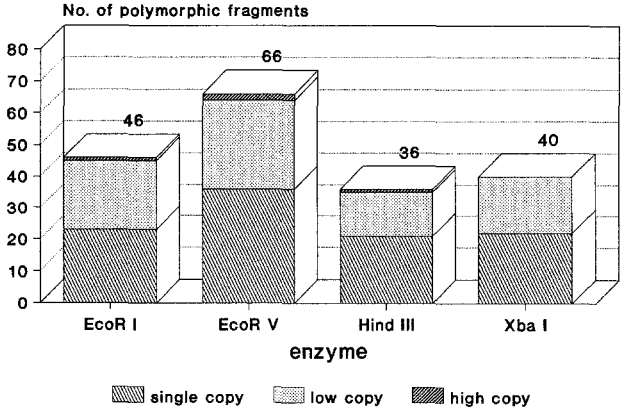

Fig. 3. Assessment of the degree of DNA polymorphism revealed by four restriction enzymes that were used for digesting sugar beet DNA; 180 polymorphic DNA fragments were included in this test. All the polymorphisms are restricted to population S227

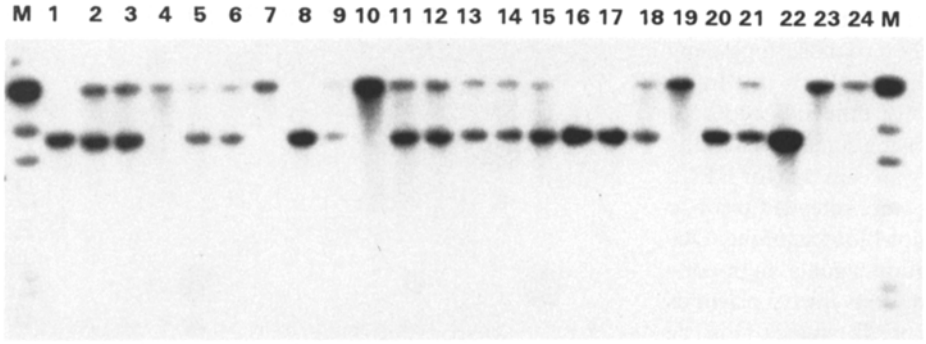

Fig. 4. RFLP polymorphism in population S227 illustrated by probe pKP557. Sugar beet DNA was digested with restriction enzyme *Eco*RV. The size marker was Lambda/*Hin*dIII (*M*). The probe reveals heterozygosity (e.g., *lane 2*) and homozygosity (e.g., *lanes 1* and *7*) for this locus. Exposure time: 4 days

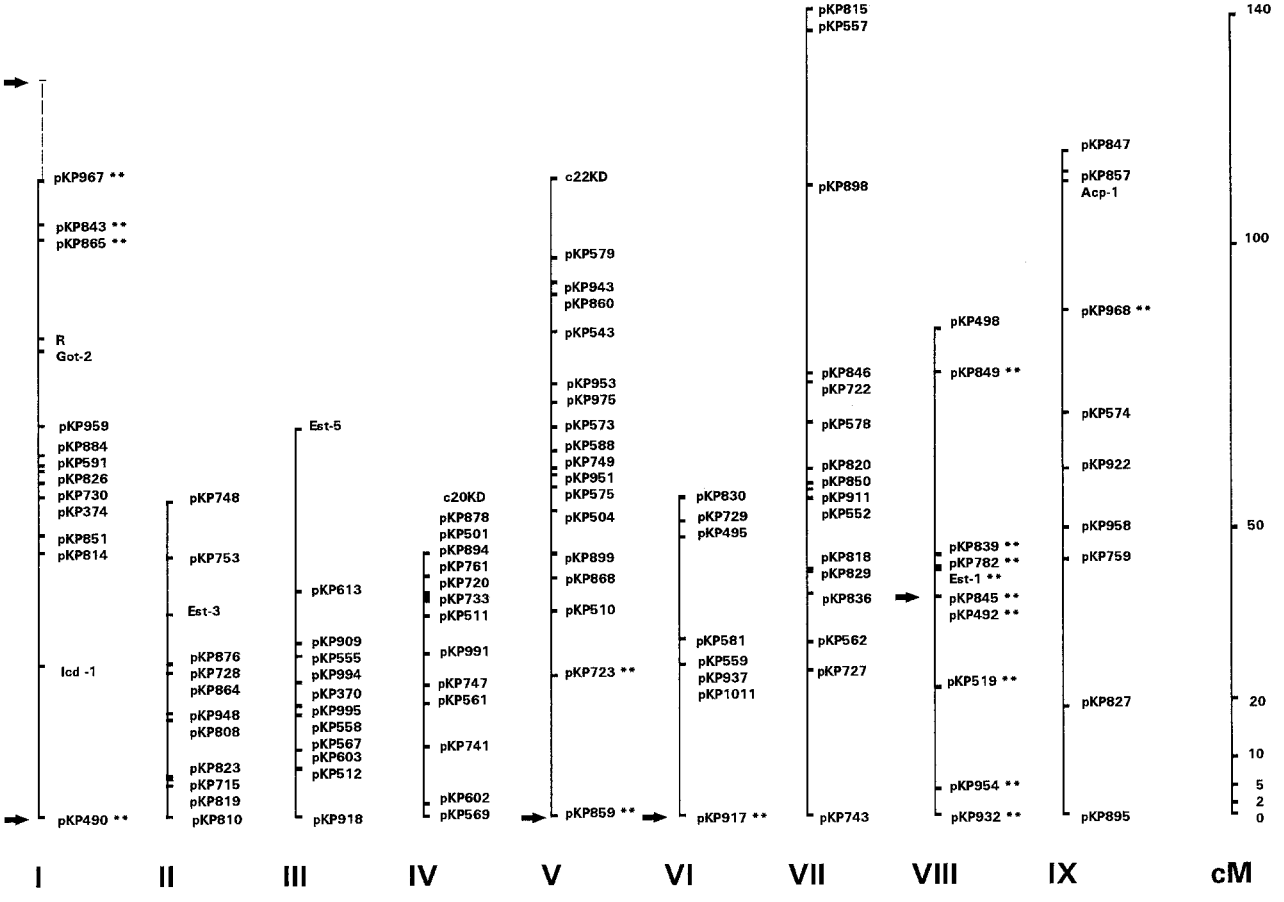

Fig. 5. Integrated map of the sugar beet genome including 1 morphological, 6 isozyme and 108 RFLP markers. *Asterisks* indicate markers with distorted segregation. Calculated positions of anonymous lethal genes are denoted by an *arrow*

Discussion

The *Beta* genome is highly enriched with repeated sequences. Also, methylation is mostly restricted to this DNA fraction (Jung et al. 1990). Consequently, cloning with a CXG methylation-sensitive restriction enzyme results in a selective enrichment of single-copy sequences cloneable into plasmid vectors. The repetitive probes still present in the *Pst*I library can be eliminated by post-cloning selection using a dot-blot technique.

Obviously, there is a high degree of DNA polymorphism within the genus *Beta*. This was to be expected because *B. vulgaris* is an outbreeding species. The population S227 is derived from an intraspecific cross between sugar beet (ssp. *vulgaris*, var 'altissima') breeding lines with no other cultured beet or wild beet in the pedigree. This implies that there is no need for mapping within interspecific offspring in this material as, for instance, in potato or tomato (Bonierbale et al. 1988) or barley (Graner et al. 1991). The low frequency of multiallelic probes corresponds to the diploid nature of this species. No conserved duplicated regions as reported, for instance, in the allopolyploid *Brassica* species (Slocum et al. 1990), were noted.

The 115 mapped marker loci are rather evenly distributed along the map. However, gaps on linkage groups I, III, VI, VII and VIII and a cluster on group IV may indicate the unequal distribution of crossover events, which may result from a clustering of repeated sequences in these regions of the genome. Inbreeding has also been shown to cause reduced chiasma frequency in outcrossing species (Lelley 1978). However, the high degree of polymorphism (47%) in the population studied here indicates a low degree of co-ancestry between the two parents. Determining the ends of the linkage groups remains a general problem as unknown genes may still be located beyond the most distal loci.

The nuclear-encoded photosystem-II genes from spinach, c20kD and c22kD, both intrinsic components of the photosystem-II reaction center (Herrmann et al. 1991) have been localized on linkage groups IV and V, respectively. Both probes display a homology to sugar beet typical of that for conserved sequences.

As karyotype analysis in *Beta* is difficult, the chromosomal relation to linkage groups is still unknown. The chromosomes are small and display only minor morphological differences. In future, we intend to use trisomic series (Butterfass 1963; Casas et al. 1989) in combination with *in situ* hybridization to localize RFLP markers to chromosomes. Alien monosomic addition lines with chromosomes from the wild species of the Procumbentes section may also be helpful in this instance (Ramos and Wricke, in preparation).

The integrated map presented here includes the limited amount of linkage data based on isozyme and morphological characters that is available. Whenever possible, our linkage groups were correlated to those previously described for classical markers. Linkage group I containing 15 markers corresponds to the linkage group I described by Smed et al. (1989) and Wagner and Wricke (1991) and the Y-R-B group (Keller 1936; Owen and Ryser 1942). In addition to the markers presented in our map, the *Est-2* and *Fdp-2* isozyme genes (Wagner and Wricke 1991), curly top resistance (C), a set of leaf colour genes, and the B gene controlling bolting behaviour are all located on this linkage group. The recombination value between *Icd-1* and R (red hypocotyl) was calculated to be 0.24, which is in fairly good agreement with previous findings (Smed et al. 1989; Wagner and Wricke 1991).

Linkage between *Got-2* and R on linkage group I was also observed by Wagner and Wricke (1991) and Abe and Tsuda (1987). The higher recombination value calculated by the latter group (0.14 versus 0.07) may result from the different plant materials studied (*B. vulgaris* × *B. vulgaris/macrocarpa* hybrids). By analyzing alien monosomic addition lines van Geyt et al. (1988) associated this linkage group with chromosome I of *B. procumbens*. However, the wild beet chromosomes are non-homologous to sugar beet chromosomes, as indicated by the absence of chromosome pairing in species' hybrids. Also, the isozyme patterns (for instance for *Icd*) are not always consistent (Van Geyt et al. 1988). Southern hybridizations with single- and low-copy sugar beet and wild beet DNA probes revealed a strikingly low degree of homology between both species (Jung and Pillen 1991).

Our linkage group II corresponds to the linkage group II postulated by Wagner and Wricke (1991). It comprises 11 DNA markers and the isozyme marker *Est-3* together with the morphological markers stem fasciation (*Verb*) and monogermy, which had not been studied in this work. The linkage groups III presented both here and by Wagner and Wricke (1991) share the *Est-5* gene. Low linkage values were computed for the restorer gene X and *Est-5* (0.31; LOD score 1.22). This gap remains to be filled with DNA markers.

In the case of complete gametic selection caused by a lethal allele in male gametes the homozygous class of a linked marker locus is reduced to zero (total linkage). The computing of linkage between this marker locus and other markers is feasible, and, in addition, estimation of the distance to the anonymous lethal locus is possible with regard to the degree of homozygous deficit (Wagner et al. 1992). For the map presented here, the calculated distances to the lethal genes are in accordance with the linkage groups. Linkage group VIII, for instance, displays a cluster of markers with distorted segregation that were localized around a hypothetical lethal locus. Distances between markers and marker positions computed by using the formula of Wagner et al. (1992) correspond to those obtained by MAPMAKER multipoint analysis. This may confirm the presence of lethal factors within the *Beta* genome previously found by Abe and Tsuda (1988) and Wagner and Wricke (1991). Deviations from expected segregations can also be caused by linkage to incompatibility loci, as was shown by Wricke and Wehling (1985) in rye. In *Beta vulgaris* 4 incompatibility loci have been analyzed (Larsen 1977). Therefore, it is necessary to map these incompatibility loci in order to be able to exclude this mechanism as a cause for deviations.

Lethal loci were localized under the assumption of complete gametic selection, indicated by the high frequency of markers with reduction of a homozygote class to zero (5 markers). However, distorted segregation may also be explained by partial gametic selection or different fitness of the zygotes. The formula used here cannot discriminate between these hypotheses. It will be a task of the future to confirm our calculations of lethal loci in other sugar beet populations.

Applications for breeding

With the strategy of cytogenetic mapping, one gene, i.e. nematode resistance, has so far been tagged in sugar beet (Jung et al., in press). Since the average distance between marker loci is relatively small, the linkage map presented will be used for tagging genes with RFLP markers. Selectable markers will be of interest to sugar beet breeders, especially with respect to characters that can be determined only with a great deal of effort as, for instance, resistances against rhizomania, *Cercospora* and nematodes. Breeding progress may also be expected from the use of selectable markers for characters that are important for hybrid seed production and which can only be scored after flowering like male sterility and monocarpic seedballs.

Since the genetic basis of sugar beet is narrow, wide crosses with wild species are generally used for introducing new genetic variability. Wild species of the genus *B. maritima* may contribute valuable characters to the breeding of sugar beet (for a comprehensive review see Van Geyt et al. 1990a), as, for instance, new male-sterile cytoplasms including restorer genes, bolting resistance, monocarpy, resistances against rhizomania and fungal diseases. Red table beets offer the possibility to improve the root shape in a way that beets with round roots can be produced that can be harvested more easily. As most of these characters are under polygenic control, it is difficult to transfer them into sugar beet because the wild species carry negative characters like bolting tendency, low sucrose content, annuality, and procumbent growth that have to be eliminated during extensive backcross programmes.

Provided that a dense map of RFLP markers is available, quantitative trait loci (QTL) can be tagged (Paterson et al. 1988), and the introduction of new characters into beets or the molecular identification of useful genes will be accelerated in the future.

Acknowledgements. The authors thank Anina Schaefer and Michael Timme for their technical assistance in RFLP analysis and Dr. Hadwig Wagner, Kaiserslautern, for the isozyme analysis.

References

Abe J, Tsuda C (1987) Genetic analysis for isozyme variation in the section Vulgares, genus *Beta*. Jpn J Breed 37:253–261

Abe J, Tsuda C (1988) Distorted segregation in the backcrossed progeny between *Beta vulgaris* L. and *B. macrocarpa* Guss. Jpn J Breed 38:309–318

Barocka KH (1962) The selection of single-germ sugar beet plants from bigerm material. I. I. R. B 25:357–367

Bennet MD, Smith JB (1976) Nuclear DNA amounts of angiosperms. Proc R Soc London B274:227–274

Bonierbale MW, Plaisted RL, Tanksley SD (1988) RFLP maps based on a common set of clones reveal modes of chromosomal evolution in potato and tomato. Genetics 120:1095–1103

Burr B, Burr FA (1991) Recombinant inbreds for molecular mapping in maize: theoretical and practical considerations. Trends Genet 7:55–60

Butterfass T (1963) Die Chloroplastenzahlen in verschiedenartigen Zellen trisomer Zuckerrüben (*Beta vulgaris* L.). Z Bot 52:46–77

Casas A, Lasa JM, Hecker RJ, Romagosa I (1989) In vitro multiplication of primary trisomic sugarbeets. J Sugar Beet Res 26:19–25

Chinoy CN, Devos KM, Bringloe D, Gray JC, Gale MD, Dyer TA (1991) Chromosomal location of the genes for ferredoxin in wheat. Theor Appl Genet 82:1–2

Coe EH, Neuffer MG, Hoisington DA (1988) The genetics of corn. In: Sprague GF, Dudley JW (eds) Corn and corn improvement. 3rd edn. American Society of Agronomy, Madison Wis, pp 81–258

Dellaporta SL, Wood J, Hicks JB (1983) A plant DNA minipreparation: Version II. Plant Mol Biol Rep 4:19–21

Ecke W, Michaelis G (1990) Comparison of chloroplast and mitochondrial DNA from five morphologically distinct *Beta vulgaris* cultivars: sugar beet, fodder beet, beet root foliage beet and Swiss chard. Theor Appl Genet 79:440–442

Feinberg AP, Vogelstein B (1983) A technique for radiolabeling DNA restriction endonuclease fragments to high specific activity. Anal Biochem 137:266–267

Fritzsche K, Metzlaff M, Melzer R, Hagemann R (1987) Comparative restriction endonuclease analysis and molecular cloning of plastid DNAs from wild species and cultivated varieties of the genus *Beta* (L). Theor Appl Genet 74:589–594

Gebhardt C, Ritter E, Debener T, Schachtschabel U, Walkemeier B, Uhrig H, Salamini F (1989) RFLP-analysis and linkage mapping in *Solanum tuberosum*. Theor Appl Genet 78:65–77

Graner A, Siedler H, Jahoor A, Herrmann RG, Wenzel G (1990) Assessment of the degree and the type of restriction fragment length polymorphism in barley (*Hordeum vulgare*). Theor Appl Genet 80:826–832

Graner A, Jahoor A, Siedler H, Schondelmaier J, Pillen K, Fischbeck G, Wenzel G, Herrmann RG (1991) Construction of an RFLP map of barley. Theor Appl Genet 83:250–256

Herrmann RG, Oelmüller R, Bichler J, Schneiderbauer A, Steppuhn J, Wedel N, Tyagi AK, Westhoff P (1991) The thylakoid membrane of higher plants: genes, their expression and interaction. In: Herrmann RG, Larkins B (eds) Plant molecular biology 2. Plenum Press, New York London, pp 411–428

Jung C, Pillen K (1992) Molecular analysis of the *Beta* genome. Report of the Second World *Beta* Network Meeting. International Board of Plant Genetic Resources, Rome (in press)

Jung C, Kleine M, Fischer F, Herrmann RG (1990) Analysis of DNA from a *Beta procumbens* chromosome fragment in sugar beet carrying a gene for nematode resistance. Theor Appl Genet 79:663–672

Jung C, Koch R, Fischer F, Brandes A, Wricke G, Herrmann RG (1992) DNA markers closely linked to nematode resistance genes in sugar beet (*Beta vulgaris* L.) using chromosome additions and translocations originating from wild beets of the Procumbentes section. Mol Gen Genet (in press)

Keller W (1936) Inheritance of some major colour types in beets. J Agric Res 52:27–38

Kosambi DD (1944) The estimation of map distances from recombination values. Ann Eugen 12:172–175

Lander ES, Green P, Abrahamson J, Barlow A, Daly MJ, Lincoln SE, Newburg L (1987) MAPMAKER: An interactive computer package for constructing primary genetic linkage maps of experimental and natural populations. Genomics 1:174–181

Landry BS, Kesseli RV, Farrara B, Michelmore RW (1987) A genetic map of lettuce (*Lactuca sativa* L.) with restriction fragment length polymorphism, isozyme, disease resistance and morphological markers. Genetics 116:331–337

Larsen K (1977) Self-incompatibility in *Beta vulgaris* L. 1. Four gametophytic complementary S-loci in sugar beet. Hereditas 85:227–248

Lelley T (1978) Genetic control of chiasma frequency and distribution in rye *Secale cereale*. Can J Genet Cytol 20:471–474

McCouch SR, Kockert G, Yu ZH, Wang ZY, Khush GS, Coffman WR, Tanksley SD (1988) Molecular mapping of rice chromosomes. Theor Appl Genet 76:815–829

Owen FW, Ryser GK (1942) Some Mendelian characters in *Beta vulgaris* L. and linkages observed in the Y-R-B group. J Agric Res 65:153–171

Paterson AH, Lander ES, Hewitt JD, Peterson S, Lincoln SE, Tanksley SD (1988) Resolution of quantitative traits into Mendelian factors by using a complete linkage map of restriction fragment length polymorphisms. Nature 335:721–726

Schmidt T, Jung C, Metzlaff M (1991) Distribution and evolution of two satellite DNAs in the genus *Beta*. Theor Appl Genet 82:793–797

Slocum MK, Figdore SS, Kennard WC, Suzuki JY, Osborn TC (1990) Linkage arrangement of restriction fragment length polymorphism loci in *Brassica oleracea*. Theor Appl Genet 80:57–64

Smed E, van Geyt JPC, Oleo M (1989) Genetical control and linkage relationships of isozyme markers in sugarbeet (*B. vulgaris* L.): 1. Isocitrate dehydrogenase, adenylate kinase, phosphoglucomutase, glucosephosphateisomerase, cathodal peroxidase. Theor Appl Genet 78:97–104

Tanksley SD (1988) Molecular mapping of plant chromosomes. In: Gustafson RAP (ed) Chromosome structure and function. Plenum Publ, New York, pp 157–173

Van Geyt JPC, Oléo M, Lange W, De Bock TSM (1988) Monosomic additions in beet (*Beta vulgaris*) carrying extra chromosomes of *Beta procumbens*. Theor Appl Genet 76:577–586

Van Geyt JPC, Lange W, Oléo M, De Bock TSM (1990a) Natural variation within the genus *Beta* and its possible use for breeding sugar beet: A review. Euphytica 49:57–76

Van Geyt JPC, Smed E, Oléo M (1990b) Genetical control and linkage relationships of isozyme markers in sugar beet (*B. vulgaris* L.): 2. NADP- and NAD-specific malate dehydrogenase, 6-P-gluconate dehydrogenase, shikimate dehydrogenase, diaphorase and aconitase. Theor Appl Genet 80:593–601

Wagner H, Wricke G (1991) Genetical control of five isozyme systems of sugar beet (*Beta vulgaris* L.). Plant Breed 107: 124–130

Wagner H, Weber WE, Wricke G (1992) Estimating linkage relationship of isozyme markers and morphological markers in sugar beet (*Beta vulgaris* L.) including families with distorted segregations. Plant Breed 108:89–96

Weihe A, Dudareva NA, Veprev SG, Maletsky SI, Melzer R, Salganik RI, Börner T (1991) Molecular characterization of mitochondrial DNA of different subtypes of male-sterile cytoplasms of sugar beet *Beta vulgaris* L. Theor Appl Genet 82:11–16

Wricke G, Wehling P (1985) Linkage between an incompatibility locus and a peroxidase isozyme locus (*Prx7*) in rye. Theor Appl Genet 71:289–291