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RFLP analysis and linkage mapping in *Solanum tuberosum*

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Summary. A morphologically and agronomically heterogeneous collection of 38 diploid potato lines was analysed for restriction fragment length polymorphisms (RFLPs) with 168 potato probes, including random genomic and cDNA sequences as well as characterized potato genes of known function. The use of four cutter restriction enzymes and a fragment separation range from 250 to 2,000 bases on denaturing polyacrylamide gels allowed the detection of RFLPs of a few nucleotides. With this system, 90% of all probes tested showed useful polymorphism, and 95% of those were polymorphic with two or all three enzymes used. On the average, 80% of the probes were informative in all pairwise comparisons of the 38 lines with a minimum of 49% and a maximum of 95%. The percentage of heterozygosity was determined relative to each other for each line and indicated that direct segregation analysis in F1 populations should be feasible for most combinations. From a backcross involving one pair of the 38 lines, a RFLP linkage map with 141 loci was constructed, covering 690 cMorgan of the *Solanum tuberosum* genome.

Key words: Solanum tuberosum – RFLP – Linkage map

Introduction

Besides direct sequence comparisons, restriction fragment length polymorphisms (RFLPs) are the most sensitive tool for the detection of DNA differences within or between species. The molecular basis of RFLPs is the loss or gain of a restriction site due to a point mutation within the enzymes recognition sequence, or a molecular event leading to insertion, deletion or inversion. Both situations result in a length difference of genomic restriction fragments detectable on Southern blots.

RFLPs are genetic markers with several advantages compared to conventional markers. They describe directly the genotype instead of the phenotype and, therefore, are not influenced by the environment. The number of RFLP markers which can be mapped is only limited by the molecular differences existing between available genotypes; it is also proportional to the effort applied to detect them. RFLP studies and detailed RFLP linkage maps are recognized as important contributions of molecular genetics to plant breeding (Beckmann and Soller 1986; Burr et al. 1983; Tanksley 1983) as well as to the fields of plant taxonomy and evolution (Song et al. 1988; Hosaka and Hanneman 1988a, b).

In recent years RFLP linkage maps have been developed in several diploid plants, the most advanced being maize (Helentjaris et al. 1986; Helentjaris 1987), tomato (Bernatzky and Tanksley 1986a; Helentjaris et al. 1986; Zamir and Tanksley 1988), lettuce (Landry et al. 1987b), rice (McCouch et al. 1988), pepper (Tanksley et al. 1988) and *Arabidopsis* (Chang et al. 1988). The cultivated potato (*Solanum tuberosum* ssp. *tuberosum*) is a tetraploid with poorly developed cytogenetics. In fact, no linkage map is available for the species, although a few linkages among isoenzymes have been obtained by Douches and Quiros (1987; see also Desborough 1983).

A comparative RFLP map between tomato and potato has been recently obtained from an interspecific cross involving the wild species *S. phureja* and *S. chacoense* and a diploid *S. tuberosum* line (Bonierbale et al. 1988). However, it seemed desirable to us to produce a RFLP linkage map within a gene pool of diploid *S. tuberosum*, where many crosses of agronomic interest can be analysed with the aim of mapping disease resistance genes or quantitative trait loci. Therefore, we conducted a survey of RFLPs present in a collection of 38 diploid potato breeding lines, which were selected for

agronomic qualities over years at the Max-Planck-Institut für Züchtungsforschung (review in Ross 1986). A RFLP linkage map has been obtained from a segregating backcross of one pair of diploid lines out of the 38 analysed.

Materials and methods

Plant material

Thirty-eight diploid *Solanum tuberosum* lines from the collection of the Max-Planck-Institut für Züchtungsforschung were selected for screening the probes. The line identifications together with their current running numbers are listed in the first two columns of Table 1. One F1 plant (H 86.0916/2) from a cross between lines 9 (H 81.691/1) and 16 (H 82.309/5) was the pollen parent in a backcross to line 16. Sixty-seven backcross lines were obtained and used for the linkage analysis.

For DNA extraction, leaves and young shoots were harvested from pot-grown plants in the greenhouse and stored at −70 °C. Freeze-dried material was also used for DNA isolation.

DNA-isolation

Total genomic DNA was purified according to Saghai-Maroof et al. (1984), with the following modifications and additional purification steps: two times concentrated extraction buffer was used for fresh (stored frozen at −70 °C) as well as freeze-dried material. Further purification steps involved either one CsCl gradient centrifugation step or a treatment with RNAse A (Boehringer Mannheim, 10 µg/ml, 30 min, 37 °C), followed by one phenol/chloroform and two chloroform extractions; contaminating carbohydrates were partially removed by adjusting the final aqueous DNA-solution to 1 *M* NaCl, incubating 20 min at −20 °C, centrifuging 15 min at 4 °C and discarding the gelatinous pellet. After a final ethanol precipitation, the DNA was resuspended in 10 m*M* TRIS HCl, 1 m*M* EDTA, pH 8.0. The concentration of CsCl-purified DNA was estimated by the absorbance at 260 nm, otherwise by band-intensity on Ethidiumbromide-stained agarose gels.

Restriction digests, electrophoresis and blotting

Genomic DNA was restricted with 3–4 units/µg DNA of TaqI, RsaI, HaeIII or AluI (Boehringer Mannheim) for 4 h or overnight, according to the supplier's instructions.

Sample preparation, electrophoresis and blotting was essentially as described by Kreitman and Aquadé (1986) with the following modifications: restriction digests containing 3–4 µg DNA were ethanol-precipitated and resuspended in 5 µl loading buffer (94% formamide, 10 m*M* EDTA, 0.05% xylene cyanol, 0.05% bromophenol blue). Undissolved material was pelleted by a short spin of the sample tubes. After denaturation (5 min, 95 °C), the sample tubes were transferred to ice and the supernatants were loaded into 3 mm wide slots of a 30 cm × 40 cm × 1 mm denaturing 4% polyacrylamide gel in 1 × TEB-buffer, 8 *M* urea. The running buffer was 1 × TEB (0.089 *M* TRIS-borate, 0.089 *M* boric acid, 2 m*M* EDTA). Electrophoresis was performed at 50 W constant power until the xylene cyanol dye reached the bottom of the gel. After electrophoresis, the gel was immediately transferred into a 30 cm × 40 cm electroblotting chamber (electrophoresis and electroblotting equipment manufactured by the MPI workshop) and blotted in 23 l 0.4 × TEB-buffer for 1 h at 5 V/cm onto a nylon membrane (Amersham Hybond N). The DNA was covalently bound to the membrane by UV irradiation at 302 nm on a transilluminator for 5–10 min.

Table 1. Characterization of 2nd potato lines by polymorphism and heterozygosity

No.	Line identification	Average informative probes (%)	(Min.-Max) (%)	Heterozygosity (LH) in %	Relative heterozygosity index (RHI)
3	H80.577/1	79.3	(74.1–89.8)	62	0.370
5	H80.649/1	79.3	(74.1–90.5)	52	0.348
6	H80.695/12	85.7	(81.6–93.2)	60	0.381
7	H81.8/1	78.2	(72.8–88.4)	22	0.298
9	H81.691/1	78.6	(71.4–93.2)	57	0.350
10	H81.2045/10	87.0	(81.0–93.2)	(1)	(1)
11	H81.2062/1	81.7	(57.8–90.5)	63	0.381
12	H81.2077/11	82.2	(66.7–90.5)	55	0.364
13	H79.0134/44	84.9	(76.9–92.5)	58	0.355
15	H82.24/3	82.0	(74.8–89.8)	48	0.348
16	H82.309/5	77.8	(66.0–91.8)	59	0.350
17	H82.310/4	76.4	(49.0–90.5)	51	0.351
18	H82.337/49	75.9	(56.5–91.8)	54	0.342
19	H82.340/18	76.6	(68.0–91.2)	50	0.353
20	H82.350/6	76.2	(55.8–93.2)	50	0.319
23	H82.379/7	77.1	(71.4–91.8)	38	0.334
27	H81.802/7	78.0	(66.0–91.2)	57	0.341
28	H81.2074/2	81.5	(57.8–91.2)	63	0.363
29	H82.328/13	82.3	(77.6–91.8)	55	0.355
30	H82.350/7	76.3	(68.0–91.8)	39	0.334
31	H75.1207/7	79.6	(71.4–91.2)	54	0.357
32	H75.1208/13	81.1	(70.7–92.5)	55	0.360
33	H76.7/7	75.3	(55.8–90.5)	44	0.310
34	H77.409/13	81.8	(72.8–94.6)	53	0.352
35	H77.420/10	78.8	(71.4–92.5)	38	0.305
37	H80.572/5	80.0	(74.1–89.6)	59	0.352
38	H80.576/16	80.0	(71.4–90.5)	50	0.332
39	H79.0136/76	82.1	(78.2–89.8)	54	0.353
40	H80.696/4	86.5	(83.7–94.6)	68	0.378
41	H79.1506/1	80.7	(76.9–92.5)	40	0.309
44	H81.404/89	79.4	(72.8–92.5)	41	0.341
45	H82.310/10	76.3	(63.3–89.1)	59	0.357
46	H82.355/7	74.7	(66.0–89.8)	51	0.324
47	H82.364/19	77.8	(66.0–92.5)	47	0.323
48	H82.366/3	78.1	(69.4–94.6)	56	0.342
49	H82.368/3	77.0	(70.1–93.9)	50	0.327
50	H82.2032/1	86.1	(77.6–94.6)	50	0.357
51	H81.1506/60	80.4	(73.0–92.7)	50	0.330
Mean value		79.8	(49.0–94.6)		

(1) This line was heterogeneous either due to the mixing of plant materials or of DNAs or due to a ploidy level different from 2n. The calculation of the LH and RHI indexes was, therefore, omitted

Probe preparation, hybridization and autoradiography

Recombinant plasmids were purified according to Birnboim and Doly (1979). Inserts of cDNA and genomic clones, were isolated by electroelution from agarose gels and labelled with ^{32}P-α dCTP (Amersham) using the random primer labelling method of Feinberg and Vogelstein (1983, 1984).

For hybridization, one to four membranes were stacked on top of each other and placed around the inner wall of a plastic tube (30 cm × 3.5 cm in diameter, Macrolon), and sealed with a silicon plug with a syringe needle in the center for pressure exchange. Eight to ten milliliters per membrane (25 cm × 30 cm)

of 6 × SSC, 5 × Denhardt's solution, 0.5% SDS, 20 µg/ml denatured salmon sperm DNA were added to the tube for prehybridization and hybridization, respectively.

The tubes were incubated at 65 °C, rotating around the long axis, in a hybridization oven (oven and tubes by Bachhofer, Reutlingen, FRG). Prehybridization and hybridization were performed overnight. Posthybridization washes were first in the tubes with 3 × 50 ml 1 × SSC, 0.1% SDS, 65 °C, 15 min each, then with 2 × 31 (for up to 10 membranes) 5 m*M* Sodiumphosphate, 1 m*M* EDTA, 0.2% SDS, pH 7.0, at room temperature for 30 min each or longer. The membranes were autoradiographed at −70 °C from 3 to 10 days using Kodak X-OMAT AR5 film and one intensifier screen.

Preparation of random cDNA clones

Poly A^+ RNA was isolated from young shoots and leaves according to Bartels and Thompson (1983). Double-stranded cDNA was synthesized following Gubler and Hoffmann (1983), ligated to EcoRI linkers and cloned into the EcoRI site of the bluescribe vector (Vector Cloning Systems, San Diego/CA). After transformation of *E. coli* strain TG-2 (Hanahan 1983) with the recombinant DNA, a library of ca. 3,500 clones was obtained. Random cDNA probes were isolated from clones with inserts of a least 200 base pairs. The nomenclature used for cDNA RFLP markers is CPn(a), CPn(b) and so on, with n being an identification number and a, b etc indicating different loci within the same probe.

Preparation of random genomic clones

Total DNA from a 2n potato line was digested with PstI as described by Young et al. (1987). The fragments were separated on a 1% agarose gel. Fragments between 500 and 2,000 base pairs were cut out from the gel, electroeluted with a Biotrap (Schleicher and Schüll) and ligated into PstI-digested bluescribe vector. Clones from the library (2,500 clones) obtained after transformation of *E. coli* strain TG-2 were screened for highly repetitive sequences according to Landry and Michelmore (1985). Random genomic probes were isolated from clones giving no hybridization signal with ^{32}P-labelled total potato DNA. The nomenclature for genomic RFLP markers is GPn(a), GPn(b) etc. analogous to cDNA markers.

Specified clones used as RFLP markers

cDNA clones coding for 4-coumarate: CoA ligase (4CL) and phenylalanine ammonia-lyase (PAL) of potato were obtained from K.-H. Fritzemeier (MPI für Züchtungsforschung, Cologne; Fritzemeier et al. 1987). Two potato cDNA clones, pC 116 and pI 471, were provided by J. Taylor (ditto). pI 471 codes for a gene involved in the defense reaction of potato against *Phytophthora infestans*. A similar function is not confirmed for pC 116 which has been isolated in a similar context (J. Taylor and G. Strittmatter, unpublished results). The cDNA clone coding for granule bound starch synthase (*Wx* gene) of potato was obtained from M. Hergersberg, MPI, Cologne (Hergersberg 1988). M. Thangavelu, Plant Breeding Institute, Cambridge, provided the genomic clone λPAc 59 (13.2 kb) containing an actin sequence of potato. A subcloned 1.1-kb EcoRI/HindIII fragment was used as RFLP marker. The cDNA clone rbcS c (Eckes et al. 1985) and genomic clones coding for rbsS 1, rbcS 2a, rbcS 2b and rbcS 2c (Wolter et al. 1988) were obtained from F.P. Wolter, MPI, Cologne. S. Rosahl (ditto) provided the genomic clone pgT 5 containing a patatin gene (Rosahl et al. 1986). Coding sequences (pR-1 and pR-2) of glutamine synthetase of *Phaseolus vulgaris* (Gebhardt et al. 1986) were used as heterologous probes for screening the potato cDNA library.

Data and linkage analysis

The presence and absence of specific restriction fragments were scored for each genotype on autoradiograms. Only clearly scorable fragments were analysed and doubtful cases were excluded from further data processing. The potato lines described in this paper were highly heterozygous. The presence of a restriction fragment could, therefore, indicate either homo- or heterozygosity for that fragment. The state of homo- or heterozygosity of parental, F1 and backcross lines was inferred from segregation data. Linkage analysis and calculation of recombination frequencies were computed according to Bailey (1961).

Each segregating fragment was first tested for distorted segregation ratio with the χ^2 test. Then for each pair of fragments (A and B), the fragment configuration was determined, depending on whether the two fragments were present in both parents (configuration AB/AB) or in only one parent (Configuration AB/00). The mixed configurations AB/A0 and AB/0B were also analysed. From the phenotype distribution it was inferred whether A and B were in coupling or repulsion. Taking into account the fragment configuration and distorted segregation ratio (if present), linkage was tested for each pair of fragments with the χ^2 test. Recombination frequencies were calculated using the "maximum likelihood" method. From the resulting matrix of recombination frequencies, linkage groups were deduced with the "nearest neighbour" method. Linkage subgroups were obtained preferentially for the fragment configurations AB/00 (coupling and repulsion) of each backcross parent and AB/AB (coupling), because these configurations generally had the smallest standard errors. The linkage subgroups which were independently derived from both parents were united by allelic fragments (defined by total linkage in repulsion). Allelic fragments, and fragments showing 100% co-segregation within the same probe (example in Fig. 5) constituted one locus. Loci were arranged in linear order according to Haldane's formula.

RFLP and linkage analysis were performed on an IBM-compatible PC with software developed by one of us (E. Ritter, unpublished results).

Results and discussion

The genetic material

After a series of experiments with several diploid potato lines as representatives of the genetic material currently used in Europe to breed superior 4n varieties, a set of 38 was chosen for RFLP analysis (Table 1, columns 1 and 2). The criteria adopted in selecting the genotypes were large variability in morphological (e.g. flower colour, tuber shape, eye depth, skin colour) and agronomic characters (e.g. yield, starch content, disease resistance). The variability among the lines can partially be ascribed to several wild or cultivated *Solanum* species participating in various degrees to the pedigrees, the most important being *S. acaule, S. spegazzinii, S. demissum, S. gourlayi, S. stenotomum, S. vernei, S. sparsipilum, S. stoloniferum* and *S. andigena*. Lines 6 (hybrid between *S. tuberosum* and *S. stenotonum*) and 40 (hybrid between *S. tuberosum* and *S. spegazzinii*) contain the highest amount of germplasm from another *Solanum* species whereas, e.g., lines 13 and 39 are possibly pure *tuberosum*, (except for early and not well-defined introductions from *S. demissum*,

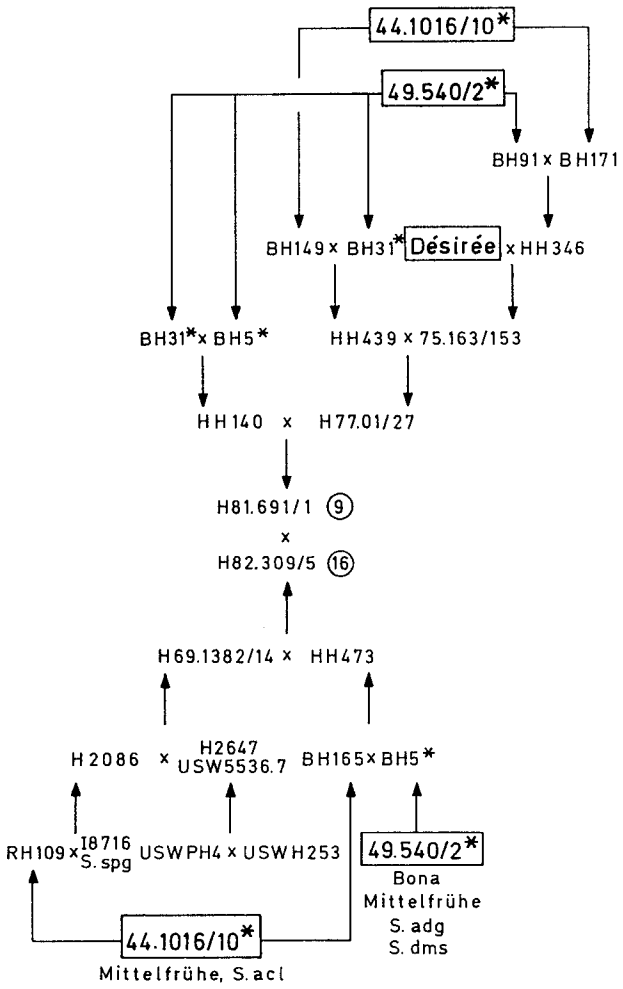

Fig. 1. Pedigrees of the 2n potato lines 9 and 16 from which the segregating backcross population was obtained. Tetraploid genotypes are shown in boxes; * indicates the repeated use of the same genotype in the pedigree. Important varieties and species contributing to the lines 44.1016/10 and 49.540/2 are indicated at the bottom. Reduction of the ploidy level from 4n to 2n occurred via female parthenogenesis induced by *S. phureja* fertilization

S. acaule and *S. andigena* into 4n varieties and breeding lines from which the diploids were later derived).

The two pedigrees of lines 9 and 16 from which the backcross for linkage analysis was obtained are shown in Fig. 1, and their complexity is rather typical for most lines included in the set. In a similar manner as lines 9 and 16, certain 4n lines and varieties are common ancestors of most of the diploid lines (Rudorf 1958: Ross and Jacobsen 1976) because of their good combining ability and valuable agronomic characters. The self-compatible line 51 was included in the set because it was used in transformation experiments by Knapp et al. (1988). Based on the pedigrees as far as known and on comparative RFLP analysis with 20 tetraploid potato varieties (Gebhardt et al. 1989), we concluded that the set of 38 diploid lines was a fairly good representation of the germplasm used in European potato breeding.

RFLP analysis within 38 diploid potato lines

From the historically narrow genetic basis of the European potato (Simmonds 1976) and from the pedigree structure of the diploid gene pool, a high variability at the DNA level could not be expected *a priori*. Therefore, we adapted the four cutter filter hybridization technique of Kreitman and Aquadé (1986) in order to improve the chances of detecting RFLPs in potato. Using four cutter restriction enzymes theoretically increases the length of sequence scored per probe by the increased number of sites (Kreitman and Aquadé 1986). Moreover, the separation range of DNA fragments between 250 and 2,000 bases allows the detection of fragment length differences as small as a few nucleotides. This is demonstrated in Fig. 2. Genomic DNA of the 38 diploid lines was digested with RsaI (Fig. 2a), HaeIII (Fig. 2b) and TaqI (Fig. 2c), respectively. The fragments were separated on a denaturing 4% polyacrylamide gel and transferred to a nylon membrane by electroblotting. The three membranes were hybridized together against the random genomic probe GP24. The length of each hybridizing fragment was determined relative to molecular weight standards and is indicated in Fig. 2. The polymorphic pattern of the probe resulted in this case from length differences of 12–15 bases.

With the experimental setup shown in Fig. 2 – except that HaeIII was replaced later on by AluI – 168 potato sequences were tested for RFLPs within the population of 38 2n lines. The probe sources were random genomic Pst clones of potato with inserts of 500–2,000 base pairs, random potato cDNA clones with inserts of at least 200 base pairs and several cloned potato genes with known or specified coding function ('Materials and methods'). Examples of patterns revealed by the probes are shown in Figs. 3 and 4. Simple patterns as in Fig. 3 were caused by single copy sequences. In Fig. 3a and b respectively, six and two allelic fragments were identified for which the homo- or heterozygous state could be determined. The percentage of heterozygosity given in Table 1 (column 5) was evaluated with this type of pattern. With one probe, null alleles were observed as shown in Fig. 3c. This finding was also reported in other species (McCouch et al. 1988; Landry et al. 1987a) and suggested a polymorphism due to insertion/deletion of DNA sequences.

In contrast to the single copy probes of Fig. 3, a very complex polymorphic pattern was revealed by the anonymous probe GP35 (Fig. 4). Whereas the most intensive hybridizing fragments showed hardly any variation, at least 23 polymorphic minor fragments were scored (indicated by arrows), taking into account only those for

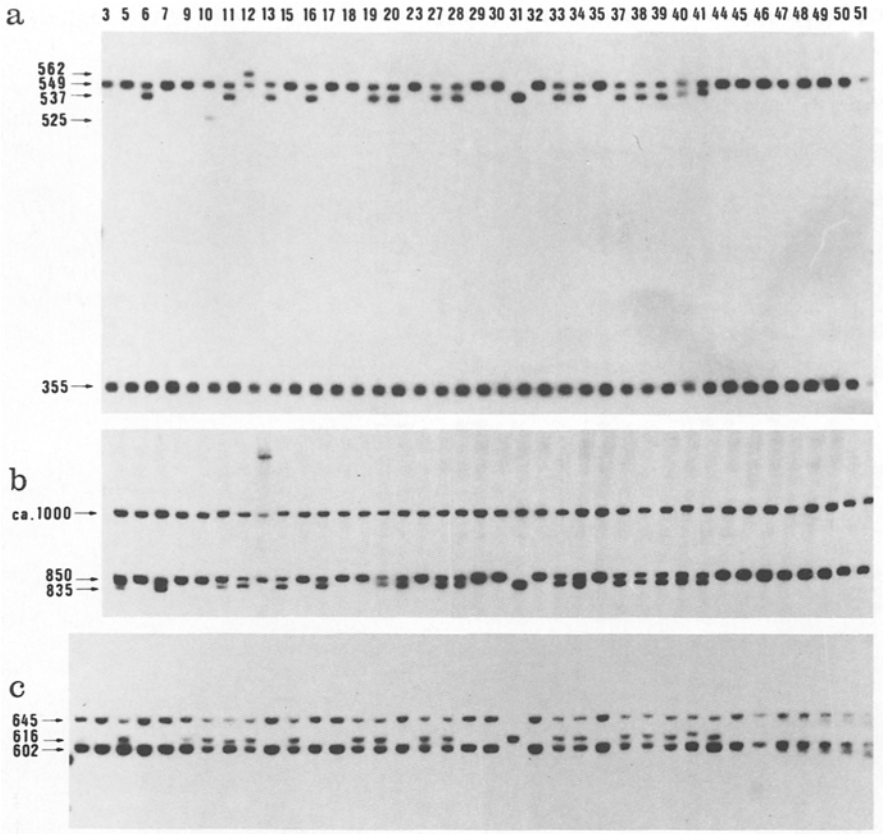

Fig. 2a–c. Southern blots of genomic DNA of 38 potato lines (numbered according to Table 1) hybridized to the genomic probe GP24. 3–4 µg DNA were restricted with **a** RsaI, **b** HaeIII and **c** TaqI respectively. The fragments were separated on a denaturing 4% polyacrylamide gel and transferred to the nylon membrane by electroblotting. Fragment lengths were determined relative to size markers (587, 540, 458, 434 and 267 bases, not shown) and are indicated to the left

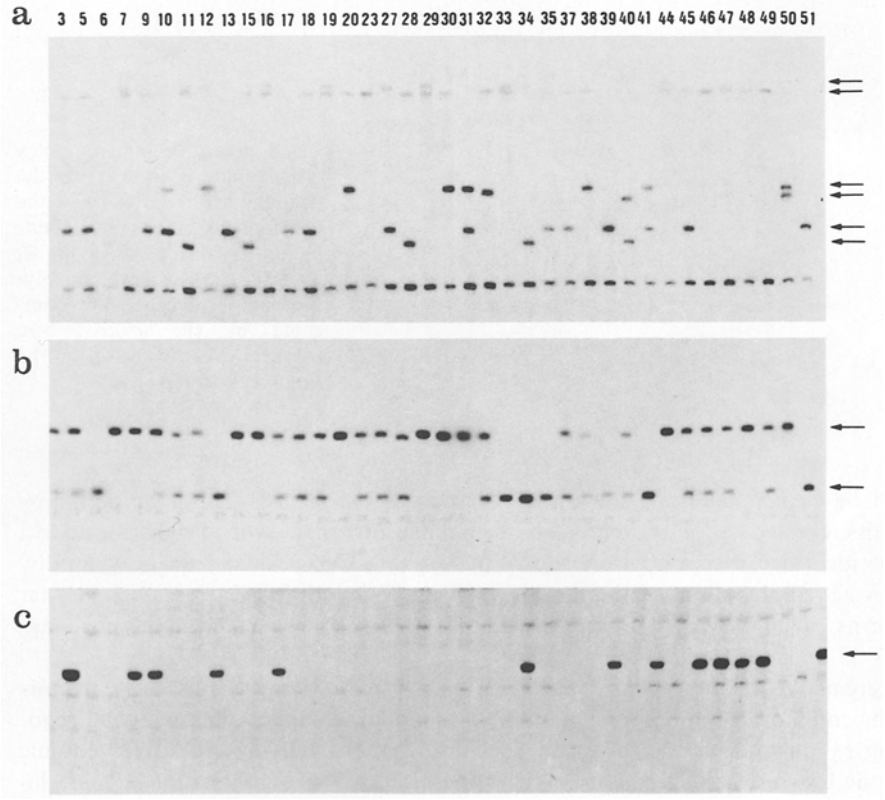

Fig. 3a–c. Examples of RFLP patterns obtained with single copy probes. Lines and experimental conditions as in Fig. 2. **a** TaqI digest, hybridized to the genomic sequence GP 79, **b** TaqI digest, hybridized to the cDNA sequence CP 57, **c** HaeIII digest, hybridized to the genomic sequence GP93. Allelic fragments are indicated by *arrows*

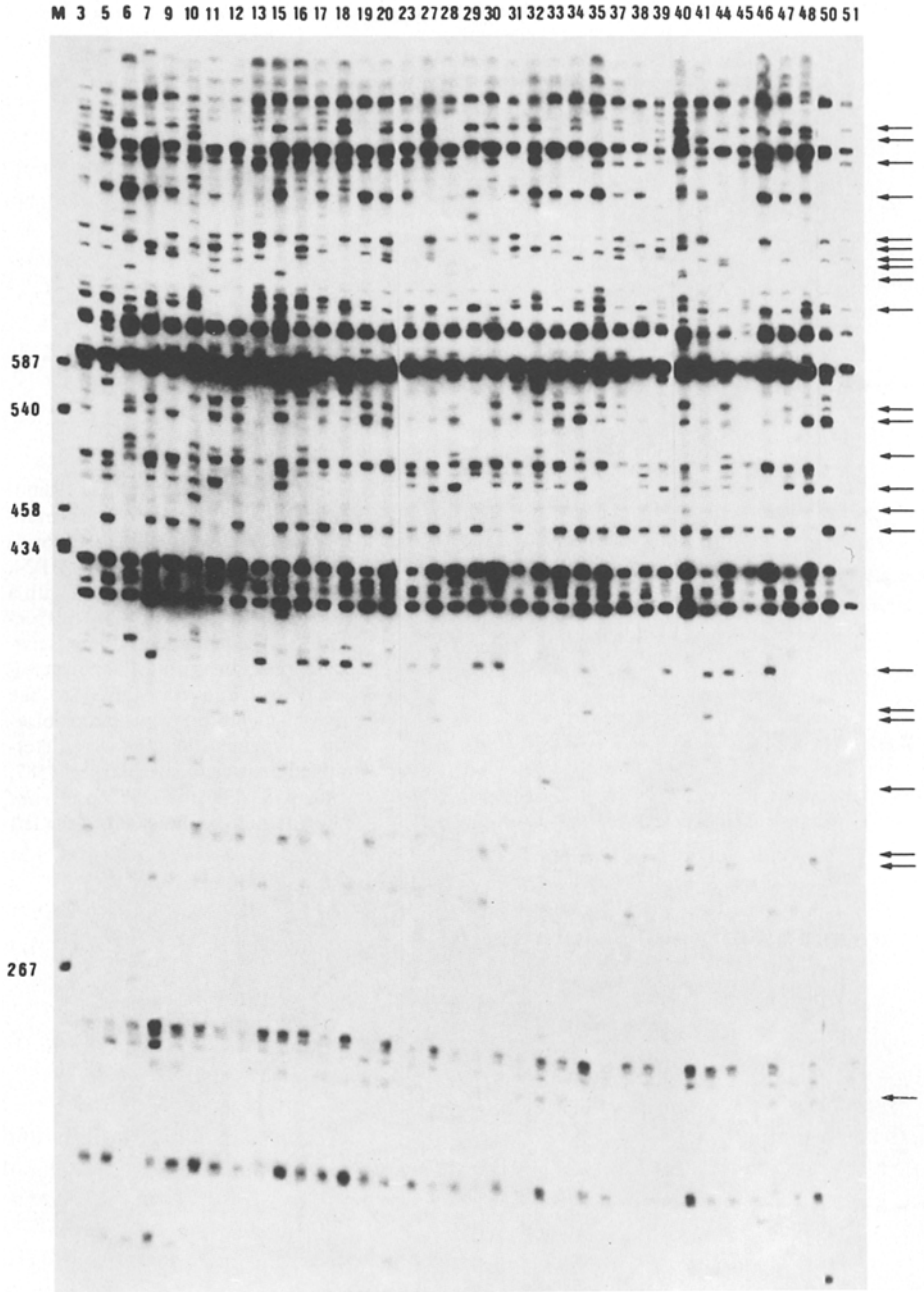

Fig. 4. Example of a complex RFLP pattern revealed by the genomic probe GP 35 with the enzyme TaqI. Lines and experimental conditions as in Fig. 2. Twenty-three scorable polymorphic fragments are indicated by the *arrows*. Size markers (M) are shown on the first track to the left

which the presence or absence could be clearly determined in most 2n lines. Allelic fragments revealed by the GP35/TaqI combination could not be identified except by linkage analysis. This probe/enzyme combination gave one of the most complex patterns of all probes tested so far and did differentiate all 38 2n lines from each other. Such a pattern is most likely derived from a multiple copy number sequence sharing common homomorphic core fragments and differing by polymorphic borders according to the position of the last restriction site in the flanking DNA sequences. This is supported by data from the linkage analysis by which eight potato loci were shown to contain GP35-related sequences, and by the fact that the probe length (604 bp) is much shorter than the length of DNA to which the numerous fragments amount.

Table 2 gives a general summary of the polymorphism found in the set of 38 diploid lines with 102 genomic and 66 cDNA probes. Highly repetitive genomic clones (ca. 5%), detected by hybridization against la-

Table 2. Polymorphism detected in 38 2n potato lines

	Genomic probes	cDNA probes
No. of probes tested	102	66
No. of probes discarded (too complex or not polymorph)	11	2
No. of probes suitable as RFLP markers	91 (100%)	64 (100%)
No. of probes		
– polymorph with 3 restriction enzymes	68 (75%)	53 (83%)
– polymorph with 2 out of 3 restriction enzymes	16 (17%)	10 (16%)
– polymorph with 1 out of 3 restriction enzymes	7 (8%)	1 (1.5%)

belled total genomic potato DNA (Landry and Michelmore 1985) are not included in Table 2. Only 13 probes, or 8%, out of 168 had to be discarded, either because they were not polymorphic with any of the three enzymes or were too complex to be analysed. The majority of the remaining 155 probes (75% of the genomic and 83% of the cDNA probes) revealed RFLPs with all three enzymes, and merely 8 (5%) with only one enzyme of the three tested. It was not possible to quantify the amount of polymorphism caused by insertion/deletion/inversion events compared to point mutations as attempted by McCouch et al. (1988), because of (1) the heterozygosity of the lines, (2) the impossibility in many cases to identify allelic fragments and (3) the use of only three restriction enzymes.

The 38 2n potato lines are the genetic material to which to concept of RFLP marker-based selection in breeding schemes (Beckman and Soller 1986) will be applied. It was therefore, of interest, to know to what extent the probe collection is informative in all possible pairwise combinations of the diploid lines. For each 2n line the percentage of probes was determined showing at least one fragment difference (with three enzymes) compared to each of the other 37 lines. Table 1 (columns 3 and 4) shows the mean value of the 37 comparisons per line, along with the minimum and maximum percentages. On the average, 80% of all probes were informative for any given combination of diploid lines with a minimum of 49% and a maximum of 95%. If the same analysis was performed with the data from only two restriction enzymes (TaqI and RsaI), only 4% of the information was lost (data not shown): on the average, 76% of probes were still informative with a minimum of 48% and a maximum of 91%. Interestingly, the minimum value was found for the comparison of two full sib lines (17×45), whereas lines 6 and 40 – being direct bastards of two *Solanum* species – belong to a group of diploids with above average differences in all comparisons. This may indicate that the frequency of RFLPs is correlated with the genetic diversity present in our materials.

The high degree of intraspecific polymorphism observed in potato can be attributed in part to the sensitivity of the experimental system used. However, variability was also easily detected with six cutter enzymes and fragment separation on agarose gels (this laboratory, unpublished), although quantitative comparisons were not performed. This suggests that intraspecific variability in the vegetatively reproduced potato is much higher than in the closely related but seed-reproduced and self-compatible cultivated tomato (Helentjaris et al. 1985). Comparable high levels of polymorphism were found so far only in *Brassica* (Figdore et al. 1988), a self-incompatible species, and maize, in which transposition was associated with the degree of polymorphism (Helentjaris et al. 1985). The high percentage of informative probes found for most pairwise comparisons within the 38 lines shows that a sufficient proportion of RFLP markers mapped with one intraspecific cross will be useful in many other crosses segregating for agronomically interesting traits.

In a subset of 46 probes and 80–93 probe/enzyme combinations, single copy patterns were obtained in which most if not all of the hybridization signal was accounted for by 1–4 fragments (Figs. 2 and 3). In these cases, it was possible to estimate the relative level of heterozygosity based on the selected sample of RFLP loci monitored by the 46 probes for each of the 38 2n lines. This relative level of heterozygosity (LH = number of probe/enzyme combinations showing heterozygosity based on their RFLP pattern, per total number of probe/enzyme combinations) is given in Table 1 (column 5). Independently, a relative heterozygosity index (RHI) was calculated by summing up the number of polymorphic fragments present per 2n line over all 155 probes and dividing the sum by the total number of scored fragment positions in the whole set. This was based on the assumption that the number of fragments present per line is positively correlated with the heterozygosity level. The RHI values are shown in Table 1 (column 6) and were highly correlated with the LH values. Line 40 – the *tuberosum* × *spegazzinii* hybrid – had, with LH = 68% and RHI = 0.378, the highest level of heterozygosity, whereas line 7 had the lowest with 22% and 0.298 respectively.

However, it should be noted that lines 3, 6, 11 and 28 also had comparatively high levels of heterozygosity similar to that of line 40, with only line 6 being a species hybrid (*stenotomum* × *tuberosum*), whereas the others were *tuberosum* × *tuberosum* hybrids, at least when the last four crosses reported in their pedigree are considered. This indicated that the level of heterozygosity present in our 2n gene pool can only be partially explained by

recent introduction of genetic material from wild *Solanum* species. From the percentage of heterozygosity – for the first time actually measured in a random sample of loci in potato – it was concluded that the F1 progeny of most crosses between the 2n lines might be sufficient for segregation analysis of RFLP markers and agronomic traits with the possible exception of line 7, which is 78% homozygous.

Linkage map

Based on preliminary RFLP data, 10 backcross populations were selected as candidates for segregation analysis to produce an RFLP map. The final choice was in favor of the backcross $16 \times (9 \times 16)$ for which 67 backcross lines were obtained using a single F1 plant as pollen parent. The segregation pattern of the anonymous genomic probe GP1, which turned out to be highly polymorphic within the 38 potato lines (Gebhardt et al. 1989), is shown in Fig. 5 as an example. Seven out of ten fragments segregated as one major locus (GP1(a)). Three fragments constituted three minor loci (GP1(b), (c) and (d)). All four loci mapped to different linkage groups (I, II, III, V, Fig. 6) and two of them, GP1(a) and GP1(c), had distorted segregation ratios.

The linkage analysis with 263 segregating fragments from 46 genomic and 43 cDNA probes placed 140 loci on 12 linkage groups equivalent to the 12 potato chromosomes (Fig. 6). Sixty-seven fragments (25.5%) showed distorted segregation ratios. Twenty-six fragments (10%) could not be mapped so far.

By heterozygosity of the parental lines, the polymorphic fragments of seven probes were lost by segregation in the F1. The loss was counteracted by segregation data obtained for two probes for which the parental lines were not polymorphic but heterozygous.

Thirty-six loci were mapped with fragments from the F1 (9×16) parent, another 36 loci with fragments from the recurrent parent 16, and 69 loci with allelic fragments from both parents. The clone GP35 (Fig. 4) identified the highest number of loci (8 loci on six linkage groups). The total map length was 690 cMorgan. The distances between markers ranged from 0 to 22.6 cMorgan with a mean distance of 5.3 cMorgan (median = 3.5 cMorgan). The present map length is, therefore, 14% longer than the 606 cMorgan given by Bonierbale et al. (1988) for an interspecific *Solanum* cross.

The small subunit of ribulose bisphosphate carboxylase of potato is coded for by five nuclear genes, three of which are tandemly repeated within 10 kb (Wolter et al. 1988). With one cDNA and two gene-specific rbcS probes (Debener, unpublished results), three rbcS loci were mapped, two to homologous positions on chromosome II as in the tomato and potato map of Bonierbale et al. 1988 (rbcS-c ≡ Rbsc1, rbcS-2 ≡ Rbcs3), and one (rbcS1 ≡ Rbcs2) to linkage group III which might, there-

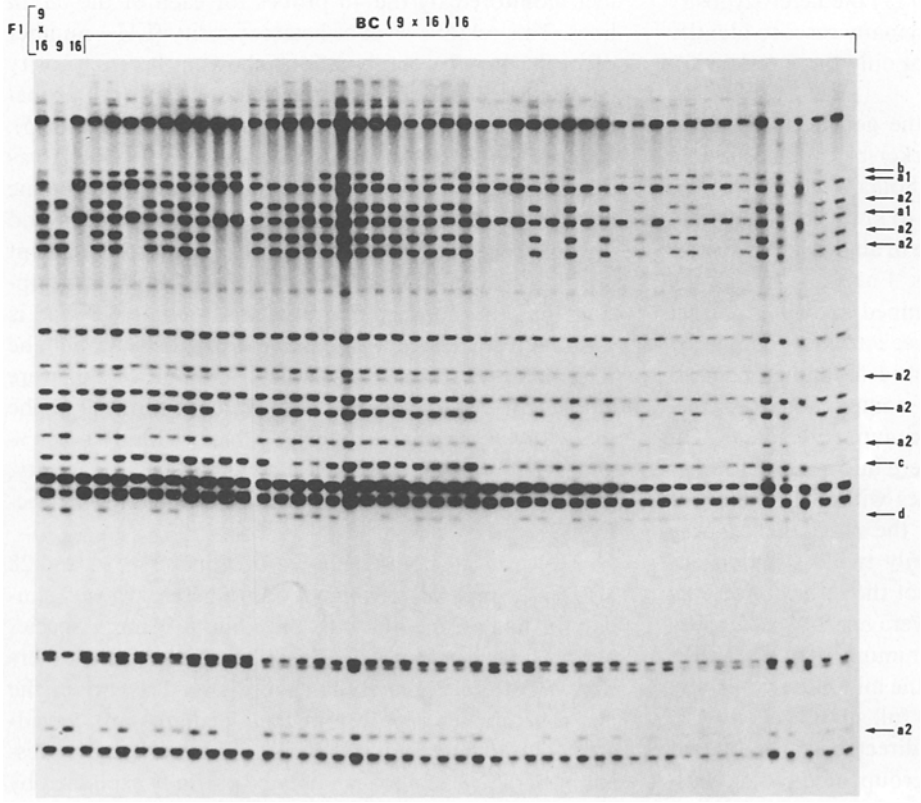

Fig. 5. Segregation analysis. Genomic DNA of lines 9 and 16 (tracks 2 and 3), the F1 parent of the backcross (track 1) and 43 backcross lines was digested with TaqI, separated and blotted as in Fig. 2 and hybridized to the probe GP 1. Fragments belonging to alleles a1 and a2 of the major locus GP 1 (a) and the fragments b, c, d defining 3 minor loci are indicated on the right

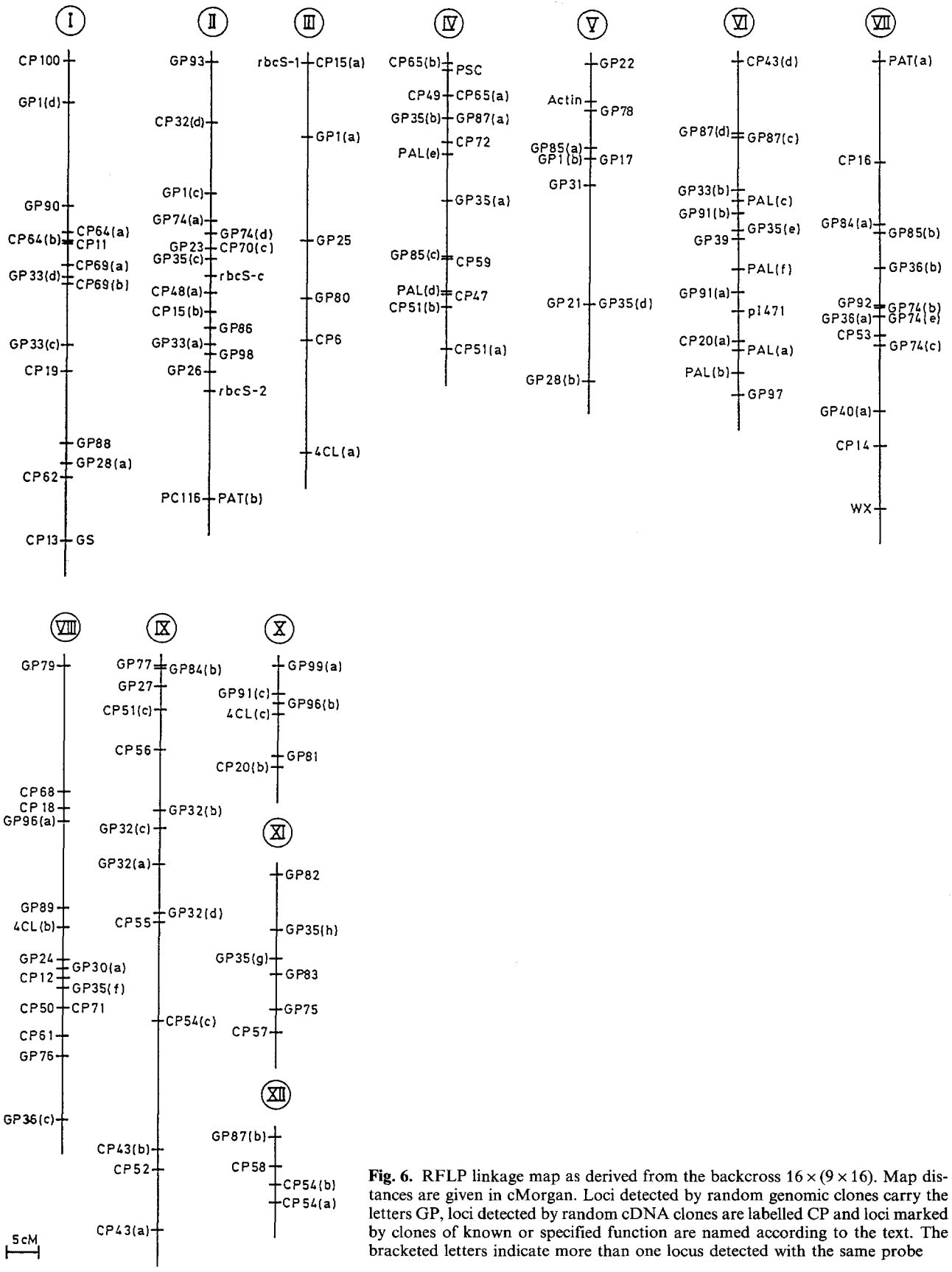

Fig. 6. RFLP linkage map as derived from the backcross $16 \times (9 \times 16)$. Map distances are given in cMorgan. Loci detected by random genomic clones carry the letters GP, loci detected by random cDNA clones are labelled CP and loci marked by clones of known or specified function are named according to the text. The bracketed letters indicate more than one locus detected with the same probe

fore, be the homologue to chromosome III in tomato (Sugita et al. 1987; Bernatzky and Tanksley 1986a).

Several map positions were obtained with potato genes known to be coded for by multigene families. About 50 gene copies were estimated for phenylalanine ammonia lyase (PAL) in potato (I. Häuser, personal communication). Six loci were identified so far, four on linkage group VI and two on IV. From the actin family (Bernatzky and Tanksley 1986b) only one locus was mapped to linkage group V. The complexity of the pattern revealed by the actin probe largely prevented the analysis. Patatin was similar with an estimated copy number per haploid genome of 12–18 (Twell and Ooms 1988). With two patatin probes from different, non-overlapping regions of the gene, two loci were mapped to linkage group VII and II. Further resolution for this class of genes might be obtained with gene specific probes and/or analysis of other segregating populations.

Map positions were also determined for the waxy gene (granule bound starch synthase, Hergersberg 1988) on linkage group VII, 4-Coumarate: CoA ligase (4Cl) (Fritzemeier et al. 1987), on linkage groups III (major locus), VIII and X (minor loci), and one presumably glutamine synthetase gene (GS) (Gebhardt et al. 1986) on linkage group I. The latter marker was a unique clone from the leaf cDNA library ('Materials and methods'), detected by hybridization to the heterologous GS sequence from *Phaseolus vulgaris*.

The backcross segregated for one morphological marker, the tuber skin colour. Approximately half of the backcross lines had purple tubers, some lines with reduced expression. The locus (PSC = purple skin colour) was mapped to linkage group IV.

In conclusion, an initial RFLP linkage map was obtained within the species *Solanum tuberosum* which should be informative in most crosses segregating for any trait of interest and, therefore, allows mapping of those traits. This was inferred from the high intraspecific variability and heterozygosity found in a representative gene pool of diploid *Solanum tuberosum* lines.

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