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High-resolution genetical and physical mapping of the *Rx* gene for extreme resistance to potato virus X in tetraploid potato

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Abstract The *Rx* locus in potato confers extreme resistance to PVX. In the F_1 progeny of crosses between the PVX-susceptible cultivar Huinkel and the cultivar Cara (*Rx* genotype) there was a 1:1 segregation of PVX resistance, indicating that *Rx* in Cara is present in the simplex condition. Using potato and tomato RFLP markers, we mapped *Rx* in Cara to the distal end of chromosome XII at a different position to the previously mapped *Rx*1 locus. To generate a high-resolution linkage map in the vicinity of *Rx* a total 728 AFLP primer combinations were screened using DNA of bulked resistant and susceptible segregants. We also screened segregating populations for chromosomal recombination events linked to the *Rx* locus and identified 82 plants with recombination events close to *Rx*. Using these recombinant plants we have identified AFLPs that flank *Rx* and span an interval of 0.23 cM in a region of the genome where 1 cM corresponds to approximately 400 kb.

Key words PVX · Resistance gene · Mapping · RFLP · AFLP · CAPS

Introduction

The *Rx* loci in potato confer extreme resistance (ER) to potato virus X (PVX) through a mechanism which, like that controlled by many disease resistance genes, can be described in terms of an elicitor/receptor model (Köhm et al. 1993; Staskawicz et al. 1995). However, there are several features of the *Rx*-mediated mechanism that are different from the virus resistance conferred by other well-characterised virus resistance genes, including the tobacco mosiac virus (TMV) resistance *N* gene in tobacco (Fraser 1990; Whitham et al. 1994). Most notably the *Rx* response is effective in the initially infected cell and is not associated with the death of infected cells (Adams et al. 1986; Köhm et al. 1993). In contrast, the resistance response of *N* and many other virus resistance genes is not active until the virus has spread into several cells and is associated with the formation of necrotic lesions around the site of inoculation (Whitham et al. 1994). These features of *Rx*-mediated resistance may indicate either that *Rx* controls an unusual resistance mechanism or else that *Rx*-mediated resistance is a variation on the type of resistance leading to cell death, in which the virus is suppressed before cell death occurs.

Until now the molecular analysis of *Rx*-mediated resistance has focussed on the virus. By mutation analysis (Kavanagh et al. 1992; Goulden et al. 1993), and using an expression vector based on TMV (Bendahmane et al. 1995), we have demonstrated that *Rx*-mediated resistance is activated by the PVX coat protein in the absence of other PVX-encoded proteins and, therefore, that the coat protein is an elicitor of the resistance mechanism (Bendahmane et al. 1995). In the present paper, we describe progress towards the isolation of the *Rx* locus.

Several plant disease resistance genes have been isolated either by transposon tagging or by the use of closely linked molecular markers. These include resistance genes with specificity for bacterial and fungal pathogens (Martin et al. 1993; Bent et al. 1994; Jones et al. 1994; Mindrinos et al. 1994; Grant et al. 1995; Lawrence et al. 1995; Song et al. 1995; Dixon et al. 1996) and, in one instance, a virus (the *N* gene conferring a hypersensitive response to TMV; Whitham et al. 1994). In principle either approach could be used to isolate *Rx* from potato. There is a well-developed

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molecular genetic map to facilitate the use of molecular markers (Tanksley et al. 1992) and it is known that maize transposons, which could be used in a tagging approach, are active in the potato genome (Jakobs et al. 1995). The tagging approach exploits the fact that transposition events occur preferentially to sites that are physically close to the transposon insertion. Thus, to adopt a tagging approach to the isolation of *Rx* it would be necessary to first identify lines with a transposon coupled to an *Rx* locus. This preliminary to gene isolation by tagging would require several crosses and would be particularly difficult with tetraploid cultivars of potato.

In contrast, the linked-marker approach has the advantage that it could use first-generation progeny of existing potato cultivars and would build on the previous demonstration that there are two *Rx* loci, on chromosomes V and XII (Ritter et al. 1991), in the potato genome. The *Rx*1 locus (chromosome XII) and *Rx*2 (chromosome V). These loci function independently of each other, have the same specificity for the PVX coat protein (Querci et al. 1995) and are probably paralogous loci introgressed into the potato genome from different *Solanum* species (Cockerham 1970). The feasibility of using a linked-marker approach for the cloning of the *Rx* locus is re-inforced by the analysis presented here describing the isolation of DNA markers that are physically and genetically close to the *Rx* locus.

Materials and methods

Plant material

F_1 seeds from the cross between the tetraploid potato cultivars Cara (*Rx*) and Huinkel (*rx*) were obtained from the Plant Breeding Institute, Cambridge. The progeny of this cross were used to determine the genotype of the individuals resistant to PVX_{CP4} Cara, to identify the chromosome position of *Rx*, and to assign RFLP markers linked to the *Rx* locus. The selfed (S_1) progeny of cv Cara (1350 plants) were used as a mapping population to locate the *Rx* locus with respect to the previously published RFLP markers (Ritter et al. 1990) and the newly identified AFLP markers. DNA from the potato varieties LT-9, DXY-7, Bzura and Atlantic, and the diploid potato P18, were kindly provided by Dr. M. Querci (CIP, Peru) and Dr. C. Gebhardt (MPI, Köln) respectively.

Test for resistance

All PVX-resistance tests used the PVX_{CP4} isolate which has been cloned as a cDNA (Goulden et al. 1993). The virus preparations employed were passaged descendants of the virus accumulating on a plant inoculated with transcripts of the cDNA clone. Potato plants were either inoculated mechanically with PVX_{CP4}, as described in Kavanagh et al. (1992), or graft-inoculated with cuttings of *Lycopersicon esculentum* cv Ailsa Craig systemically infected with PVX_{CP4}. The presence or absence of PVX_{CP4} in the total RNA of systemically infected tissue was used as an indicator of the susceptible (*rx*) or resistant (*Rx*) genotypes. Total RNA was isolated from potato leaves distal to the site of inoculation 3–4 weeks post-inoculation and transferred to Hybond-N membranes (Amersham) using a slot-blot apparatus (Bio-Rad) as described by Goulden et al. (1993). The PVX_{CP4} RNA was detected by hybridisation with a PVX-specific probe as described in Chapman et al. (1992).

Preparation of potato DNA for RFLP and AFLP analysis

Potato leaf tissue (7–10 g) was ground to a powder in the presence of liquid nitrogen, mixed with 30 ml of extraction buffer (100 mM Tris-HCl pH 8.0, 500 mM NaCl, 50 mM EDTA, 1.25% SDS, 8.3 mM NaOH, 0.38% sodium bisulphite and 0.38% sodium diethyldithiocarbamate) that had been pre-heated to 65°C, shaken well, and incubated for a further 30 min at 65°C. Most of the proteins and polysaccharides were removed by centrifugation following the addition of 10 ml of ice-cold 5 M sodium acetate, and incubation of the mixture on ice for 30 min. The supernatant was filtered through Miracloth, mixed with 0.7 vol of *iso*-propanol, and the precipitated DNA was recovered by centrifugation. The DNA pellet was dissolved in TE-buffer, pH 8.0, and purified further by CsCl density gradient centrifugation in the presence of ethidium bromide (Sambrook et al. 1989).

Preparation of potato DNA for PCR analysis

Small leaf disks, about 5 mm in diameter, were homogenised in Eppendorf tubes with 100 µl of the extraction buffer (0.22 M Tris-HCl pH 8.0, 0.022 M EDTA, 0.8 M NaCl, 0.14 M sorbitol, 0.8% CTAB, 1% *n*-lauroylsarcosine). Chloroform (100 µl) was added, and the mixture was incubated at 65°C for 5 min. Following centrifugation for 5 min in a microfuge, the supernatant was transferred to a fresh Eppendorf tube, and the DNA was precipitated with 100 µl *iso*-propanol at room temperature for 15 min. The DNA was recovered by centrifugation for 10 min, washed with 70% ethanol, air dried, and re-suspended in 50 µl of sterile deionized H_2O.

Pulsed-field gel electrophoresis and Southern analysis

High-molecular-weight DNA was prepared from cv Cara protoplasts isolated as described in Köhm et al. (1993). Protoplasts were mixed with an equal volume of 1.2% low-melting-point agarose (FMC), and 240-µl agarose plugs each containing $2.5–3.0 \times 10^6$ protoplasts (corresponding to about 8 µg of potato DNA) were prepared. The plugs were processed using the protocol of Ganal and Tanksley (1989). Half of a DNA plug (approximately 4 µg) was digested overnight with 40 units of a rare-cutting restriction enzyme: (*Sma*I, *Sal*I, *Sac*II). The DNA was fractionated by pulsed-field gel electrophoresis (PFGE) in a CHEF DRII gel apparatus (Bio-Rad) as described by Chu et al. (1986). After electrophoresis the gel was irradiated with 400 mJ of ultra-violet light using a UV Stratalinker 2400 (Stratagene) to nick high-molecular-weight DNA. The DNA in the gel was de-natured with 0.4 M NaOH, 1.5 M NaCl for 30 min, neutralised with 0.5 M Tris-HCl pH 7.5, 1.5 M NaCl for 30 min and transferred onto a Hybond-N membrane in 20 × SSC for 24 h. DNA was cross-linked to the membrane in the UV Stratalinker 2400 (Stratagene) and the membrane was baked for 60 min at 80°C. The membrane was hybridised as described in Church and Gilbert (1984).

RFLP, AFLP and CAPS markers

RFLP analysis was performed essentially as described by Gebhardt et al. (1991). RFLP markers chosen for the mapping of *Rx* in cv Cara were from tomato and potato chromosomes V and XII and were

kindly provided by C. Gebhardt and S. Tanksley (Gebhardt et al. 1991; Tanksley et al. 1992; Paul et al. 1994). The RFLP markers selected from chromosome V were CT242, CT167, TG623, TG441, and TG432. The RFLP markers selected from chromosome XII were CD6A, CT99, TG360, CT100, CT129, GP34 and CP60. To simplify the mapping, RFLP markers were also converted to CAPS (cleaved amplified polymorphic sequences; Konieczny and Ausubel 1993). The primers used for CAPS of RFLP markers are shown in Table 1.

AFLP analysis (Vos et al. 1995) was performed on bulked DNA samples of resistant and susceptible plants (Giovannoni et al. 1991; Michelmore et al. 1991) according to Keygene N.V. (Wageningen, The Netherlands) instructions using *Pst*I and *Mse*I restriction enzymes, biotinylated *Pst*I- and non-biotinylated *Mse*I-adapters, and a set of primers corresponding to the *Pst*I- and *Mse*I-adapter with two or three selective nucleotides at the 3′-end, respectively. A total of 13 *Pst*I + 2 (2 selective bases)- and 56 *Mse*I + 3 (3 selective bases)-primers were employed in a total of 728 combinations. To convert AFLP markers to CAPS the AFLP products were excised from the gel in which they were originally detected, reamplified by PCR using the original AFLP primers, and cloned into a pGEM-T vector (Promega). The sequence of the cloned fragment was used to derive primers for IPCR and the sequence of the IPCR product was used to design CAPS primers. The sequences and restriction enzymes used for CAPS derivatives of the AFLP markers are shown in Table 1.

Results

Chromosomal position of the *Rx* locus in cv Cara

Potato cv Cara carrying *Rx* was crossed to the susceptible cv Huinkel to generate an F_1-segregating population. Forty seven F_1 progeny were screened for resistance against PVX_{CP4}. Twenty five plants were resistant against PVX_{CP4} and 22 were susceptible, which is consistent with the 1 : 1 segregation ratio expected if the resistant parent carried a single dominant gene in a simplex condition. Thus, the deduced genotype of cv Cara is *Rx*, *rx*, *rx*, *rx*.

To determine whether *Rx* in Cara was linked to the previously characterised *Rx* loci in potato (Ritter et al. 1991) specific RFLP probes from tomato and potato chromosomes V and XII were tested for linkage to *Rx* in Cara. Genomic DNA from 23 members of the *Rx*-segregating population was extracted, digested with 4-bp-cutting restriction enzymes and Southern-blot hybridization was carried out using RFLP probes. There was no linkage of *Rx* to RFLP markers selected from chromosome V (data not shown). In contrast there was linkage with the CT99 RFLP marker specific for tomato chromosome XII (Fig. 1A) indicating that Cara carries *Rx* on chromosome XII as reported for the *Rx*1 locus mapped by Ritter et al. (1991). However, the tomato marker CT99 had not previously been mapped in the potato genome. To confirm that *Rx* in Cara is located on chromosome XII it was mapped relative to marker GP34 analyzed as a CAPS that is close to *Rx*1 (Ritter et al. 1991). In a population of 47 plants there was complete co-segregation of GP34, *Rx*, and CT99 (Fig. 1 B). This result suggested that *Rx* in Cara is most

Table 1 CAPS markers linked to the *Rx* locus. For each CAPS marker the sequnce of the primers, the PCR conditions and the restriction enzyme used to detect polymorphism are shown

Marker	Primers	PCR conditions	Restriction enzyme
CT99	5′-GAACCAAGGTAAACCACCACAAG 5′-GCCGGAGACATTGCCGGAGCTA	94°C, 15 s ‖ 62°C, 15 s ‖ × 35 cycles 72°C, 1 min ‖	*Rsa*I
GP34	5′-CGTTGCTAGGTAAGCATGAAGAAG 5′-GTTATCGTTGATTTCTCGTTCCG	94°C, 15 s ‖ 62°C, 15 s ‖ × 35 cycles 72°C, 1 min ‖	*Taq*I
CP60	5′-CAGCCTACCGCGAAAGTGCCTTCG 5′-GCCAACCCCACGAGTTTCTCACTGAC	94°C, 15 s ‖ 58°C, 15 s ‖ × 35 cycles 72°C, 1 min ‖	*Dde*I
CT100	5′-GCCTCAGCTGGTAATTTCTATAGAG 5′-GAGGAAACCTCTTAGAATCAGCACAG	94°C, 15 s ‖ 62°C, 15 s ‖ × 35 cycles 72°C 1 min ‖	*Sau*3A
CT129	5′-GTCTAAGAAGATGAAAAGGGGTGC 5′-TTGGAGTTTGTTAAGGACTTCGATTGC	94°C, 15 s ‖ 52°C, 15 s ‖ × 35 cycles 72°C, 1 min ‖	*RsaI*
IPM3	5′-AGTAGTTTCAGGCTAGTG 5′-CAACATCACTTGATCAGAC	94°C, 15 s ‖ 54°C, 15 s ‖ × 35 cycles 72°C, 1 min ‖	*DdeI*
IPM4	5′-GTACTGGAGAGCTAGTAGTGATCA 5′-ACCACTGGCAAATGGCCATACGA	94°C, 15 s ‖ 65°C, 15 s ‖ × 35 cycles 72°C, 1 min ‖	*Taq*I
IPM5	5′-AGCTCCATTCGTGACGAT 5′-AGCTTCGATAATTCTAAATTTG	94°C, 15 s ‖ 56°C, 15 s ‖ × 35 cycles 72°C, 1 min ‖	*Dde*I

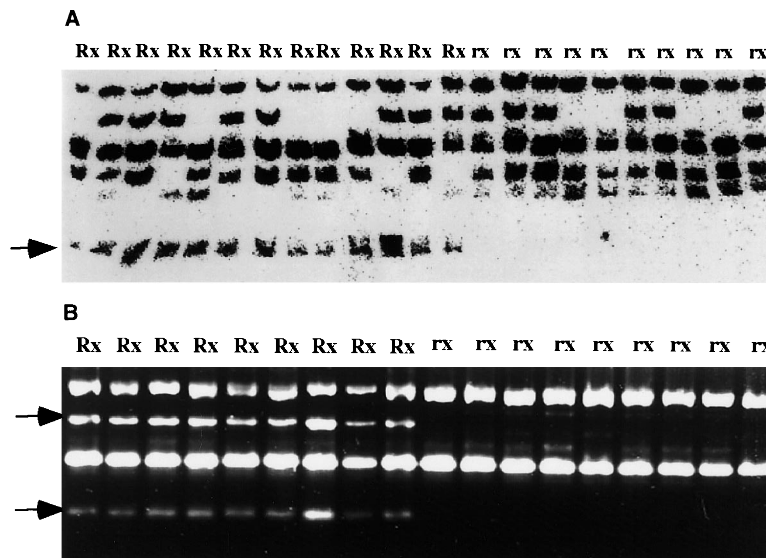

Fig. 1A, B Analysis of a segregating population of progeny of the cross Cara-Huinkel. **A** DNA from resistant (*Rx*) and susceptible (*rx*) segregant plants was digested with the *Rsa*I restriction enzyme and Southern-blot hybridisation was carried out using a CT99 tomato RFLP marker as a probe. The DNA fragment linked in *cis* to the *Rx* locus is marked by an *arrow*. Each *lane* represents DNA from a different F_1 segregant. **B** PCR was carried out on DNA from individual resistant (*Rx*) and susceptible (*rx*) segregant plants. The PCR products were digested with the *Taq*I restriction enzyme and fractionated on a 2% agarose gel to identify the DNA fragment linked in *cis* to the *Rx* locus (*arrow*)

likely in the same position as, or at least in the vicinity of, the *Rx*1 locus previously mapped to the distal end of chromosome XII by Ritter et al. (1991). We also mapped the CD6A tomato RFLP marker to *Rx*. In tomato, CD6A is 0.8 cM from CT99 (Tanksley et al. 1992). By contrast, in Cara this marker is 23.5 cM from CT99, GP34 and *Rx* (4 recombinants out of 17 plants tested) (Fig. 2A). This variation in the distance between markers indicates that, as reported previously for diploid potato, there is an inversion in chromosome XII of Cara relative to the homologous region of the tomato genome (Fig. 2B).

A series of RFLP markers were selected based on their telomeric position in potato chromosome XII (GP34 and CP60), or their centromeric position in tomato chromosome XII (CT99, TG360, CT100 and CT129), and were tested for linkage to *Rx*. To simplify the mapping, the RFLP markers were converted to CAPS markers (Konieczny and Ausubel 1993). In an F_1 population of 370 progeny of the cross Cara-Huinkel there were 15 recombinants in the interval defined by these markers. The *Rx* locus segregated between CT129 and the co-segregating CT99 and CP60 markers. GP34 and CT100 co-segregated and were positioned between the *Rx* locus and CT99.

To orientate the CT129–CT99 interval relative to the centromere, plants with recombination events in this interval were analyzed with the CD6A RFLP marker. In this analysis the CD6A and CT129 markers co-segregated in the eight recombinant plants tested (data not shown). Thus, CT129 is centromeric, and GP34, CT100, CP60 and CT99 are telomeric, with respect to *Rx* (Fig. 2B). This analysis therefore confirms that *Rx* of Cara and *Rx*1 mapped by Ritter et al. (1991) are in similar, but not identical, positions on the potato genome: the *Rx*1 locus is on the telomeric side of marker GP34 (Ritter et al. 1991) whereas the *Rx* locus in Cara is on the centromeric side.

Construction of a high-resolution-map of the *Rx* locus

Of the markers described above, the closest to *Rx* in Cara was GP34 (0.81 cM) (Fig. 2B). To identify markers more closely linked to the *Rx* locus we employed AFLP technology (Vos et al. 1995) in conjunction with a bulked segregant analysis (Michelmore et al. 1991) using DNA pools of 20 resistant and 20 susceptible plants from the progeny of Cara-Huinkel. Each AFLP primer combination displayed, on average, 70 DNA fragments (Fig. 3A). Therefore, using 13 *Pst*I + 2- and 56 *Mse*I + 3-primers in 728 combinations, approximately 50 000 loci were inspected for polymorphism. Of these, a total of 57 primer combinations identified AFLP markers linked to *Rx*.

To develop a high-resolution map in the vicinity of *Rx*, it was necessary to map these AFLP markers relative to the *Rx* locus and to each other. This high-resolution mapping was carried out in several stages. First, using CT99 and CT129 CAPS markers, 1350 plants from the selfed progeny of cv Cara were screened. This resulted in the identification of 67 individual plants containing recombination events in the

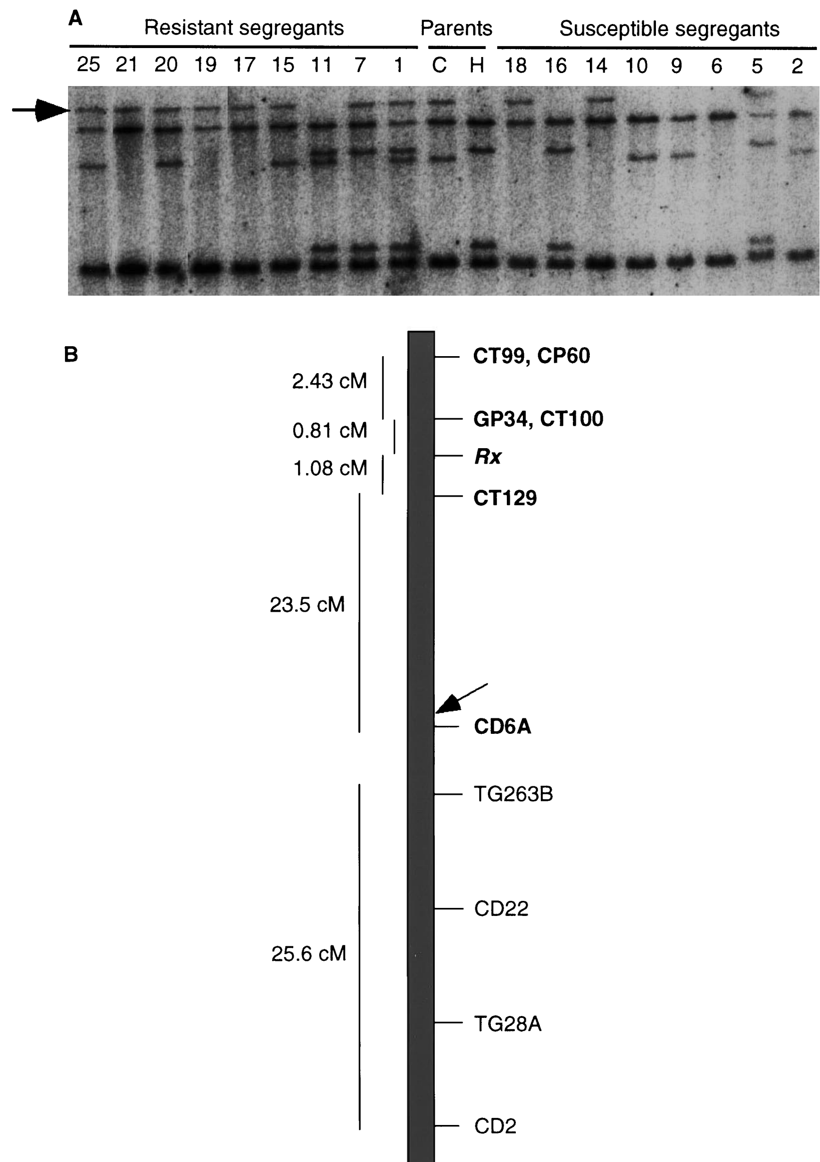

Fig. 2A, B RFLP analysis of an *Rx* segregating population with the CD6A tomato marker. **A** DNA from resistant (*Rx*) and susceptible (*rx*) segregant plants and from the cultivars Cara (*C*) and Huinkel (*H*) was digested with the *Eco*RV restriction enzyme and Southern-blot hybridisation was carried out using the CD6A tomato RFLP marker as a probe. The DNA fragment linked in *cis* to the *Rx* locus is marked by an *arrow*. There are three susceptible plants (*lanes 5, 14 and 18*) and one resistant plant (*lane 11*) with 1 recombination event between CD6A and the *Rx* locus out of 17 plants analyzed (23.5 cM). **B** Orientation of the CT129–CT99 genetical interval relative to the centromere. The data are based on 370 plants from the cross Cara-Huinkel. Map distances are given in centimorgans (cM). The relative distance between the marker in *bold* is based on 370 plants from the cross Cara-Huinkel. The RFLP markers in *plain text* were previously published (Tanksley et al. 1993). The break point of the inversion is marked by an *arrow*

interval between the markers CT99 and CT129. The recombinant plants were tested for resistance to PVX_{CP4}. Based on the outcome of the resistance test, recombination events were mapped either between CT99 and *Rx* or between *Rx* and CT129. The recombinant plants were also analyzed with the CAPS markers CP60, CT100 and GP34. Notably, crossover events were identified between markers that co-segregated in the 370 plants from the progeny of Cara × Huinkel and the CT99–CT129 interval was subdivided as shown in Fig. 3 B. The second phase in the high-resolution mapping was to position the 57 *Rx*-linked AFLP markers relative to these subdivions of the CT99–CT129 interval by an analysis of plants with recombination in the interval CT99–CT129. The outcome of this mapping is the identification of a 0.23-cM interval containing *Rx* that is flanked by AFLP markers PM3 and PM4 (Fig. 4). To confirm the AFLP map, and also to develop more easily used markers, the PM3, PM4 and PM5 markers were converted to CAPS. These new markers, designated IPM3, IPM4 and IPM5 (Fig. 5), were mapped in the interval CT99–CT129 at the same positions as the corresponding AFLP markers (Fig. 4).

Analysis of *Rx*-linked markers in *Solanum* species

There are various sources of *Rx* in European and American potato cultivars including USDA accession 41956 (which originates from the Chilean cv Villaroela of ssp. *tuberosum*), *S. tuberosum* ssp. *andigena* genotype

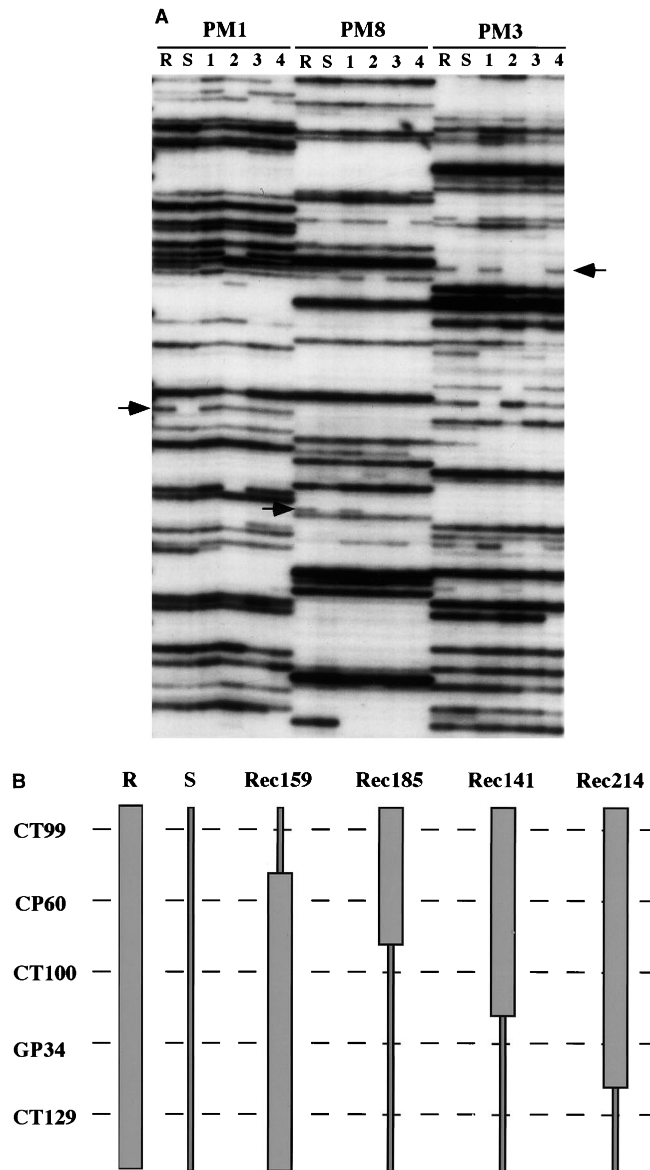

Fig. 3A, B AFLP analysis of potato DNA, **A** AFLP was carried on DNA from bulked-resistant (*R*) and bulked-susceptible (*S*) plants and from the individual recombinant plants Rec159 (*1*), Rec185 (*2*), Rec141 (*3*) and Rec214 (*4*) respectively. The PCR products were analysed on a 4.5% denaturing polyacylamide gel. The AFLP bands PM1, PM8 and PM3 linked in *cis* to the *Rx* locus are marked by an *arrow*. **B** Schematic representation of the DNA close to *Rx* in the recombinant plants used in the mapping of the AFLP markers relative to *Rx* and to CT99, CP60, CT100, GP34 and CT129 CAPS markers. The *thick and thin bars* represent DNA from resistant (*R*) and susceptible (*S*) chromosomes respectively. Rec159, Rec185, Rec141 and Rec214 are plants carrying a recombination event in the intervals CT99–CP60, CP60–CT100, CT100–GP34 and GP34–CT129 respectively

CPC 1673, *S. vernei* backcross hybrid 62-33-3, and *S. acaule* (Bradshaw and Mackay 1994). The origin of *Rx* in cv Cara is uncertain although there are data indicating that it is likely to have originated from *S. tuberosum* ssp. *andigena* genotype CPC 1673 (Dr. H. W. Kehoe, Oak Park Research Centre, Ireland; personal communication). To directly assess the likely origin of the *Rx* locus in cv Cara, genomic DNA of various potato cultivars with extreme resistance to PVX introduced from *S. tuberosum* ssp. *andigena* (Rx_{adg}) or *S. acaule* (Rx_{acl}) were analyzed with IPM4 which is 0.06 cM from the *Rx* locus in cv. Cara (Fig. 4). This marker was also present in the DNA of potato varieties LT-9 (Rx_{adg}), DXY-7 (Rx_{adg}), and Atlantic (Rx_{adg}), but not in the variety Bzura (Rx_{acl}) (Fig. 6). These data are consistent with the originally proposed origin of *Rx* in cv Cara from *S. tuberosum* ssp. *andigena*. Interestingly, the allele of the IPM4 marker linked to the *Rx* locus in cv Cara was not detected in the diploid potato clone p18 (H82.337/49) that carries the *Rx*1 locus previously mapped to the top of chromosome XII (Ritter et al. 1991).

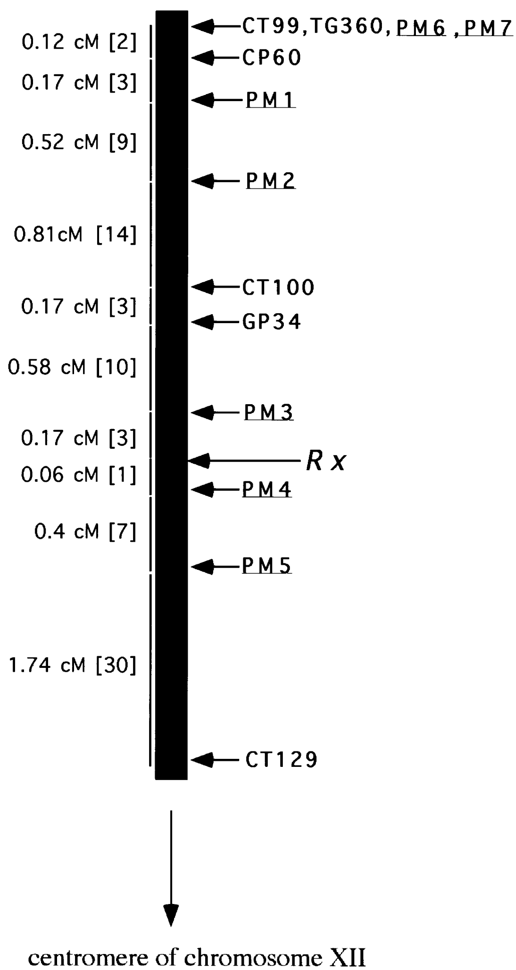

Fig. 4 High-resolution genetic map of the *Rx* locus on potato chromosome XII. The data are based on the screening of 728 AFLP primer combinations for AFLP markers linked to the *Rx* locus and 1720 segregant plants (370 plants from the cross Cara-Huinkel and 1350 plants from selfed Cara) for recombination events in the interval CT99–CT129. *PM1–PM7* are AFLP markers. Map distances are given in centimorgans (cM) and represent the percentage of recombinant plants in the 1720 plants analysed. The *numbers in square brackets* indicate the number of recombinant plants per 1720 plants tested

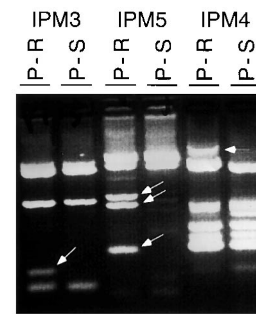

Fig. 5 IPM3, IPM4 and IPM5 CAPS markers. PCR was carried out on DNA from resistant (*P-R*) and susceptible (*P-S*) bulked-segregant plants. The PCR products were digested with *Dde*I for the IPM3 and IPM5 CAPS markers and with *Taq*I for the IPM4 CAPS marker in PCR buffer, and fractionated on a 1.2% agarose gel. The DNA fragments linked in *cis* to the *Rx* locus are marked by an *arrow*

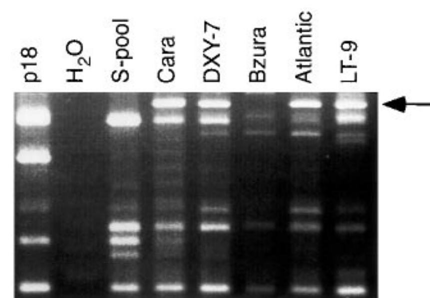

Fig. 6 Analysis of genomic DNA from different potato cultivars with the IPM4 CAPS marker. PCR was carried out on DNA from the potato varieties LT-9 (Rx_{adg}), DXY-7 (Rx_{adg}), Atlantic (Rx_{adg}), Bzura (Rx_{acl}), and in the diploid potato clone H82.337/49 (P18) that carries the *Rx*1 locus previously mapped to the top of chromosome XII but on the telomeric side of the GP34 RFLP marker (Ritter et al. 1991). The DNA fragment linked in *cis* to the *Rx* locus is marked by an *arrow*

Estimation of physical distance in the vicinity of *Rx locus*

In the high-resolution genetical map described above we positioned the *Rx* locus in an interval of about 0.23 cM between the AFLP markers PM3 and PM4 (Fig. 4). To determine the relationship between genetical and physical distances in the vicinity of *Rx*, high-molecular-weight DNA was isolated from Cara protoplasts and digested with rare-cutting restriction enzymes. Restriction fragments were fractionated by CHEF gel electrophoresis, blotted and hybridised consecutively with CT100, GP34, IPM3 and IPM4 probes (Fig. 7). This analysis revealed several co-migrating DNA fragments that hybridised to adjacent markers in the vicinity of *Rx*. These include *Sac*II fragments of 650 kb and 750 kb that hybridised to both CT100 and GP34, *Sal*I fragments of 180 kb, 220 kb and 450 kb hybridising to the same two probes, and an 80-kb fragment that hybridised to both IPM3 and IPM4. Most notably there was a 400-kb *Sma*I fragment that hybridised to all four probes tested (Fig. 7). These data are not a conclusive indication of the relationship between physical and genetical distance in the vicinity of *Rx* in cv Cara because there was no confirmation that the fragments hybridising to the different probes were the same. Nor was it established in this analysis whether the probes were detecting the chromosomal fragments carrying *Rx* or *rx*. However, we consider these data as an indication that the genetical distances in the vicinity of *Rx* correspond to physical distances that are similar to, or less

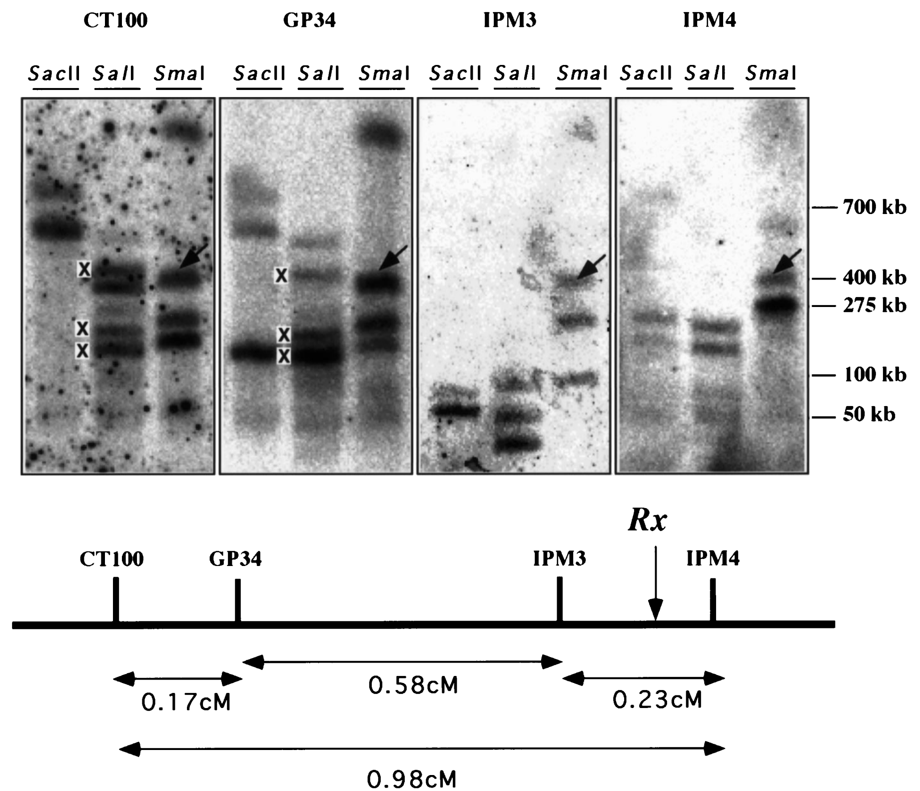

Fig. 7 Estimation of the physical distance between the CT100 and PM4 markers. High-molecular-weigh DNA from cultivar Cara was digested with rare-cutting restriction enzymes and fractionated by PFGE in a CHEF gel apparatus (Bio-Rad). The Southern hybridisation with the CT100, GP34, IPM3 and IPM4 markers was carried out as described in Materials and methods. The same filter was used for each hybridisation. The previous probe was stripped after use, and the membrane was exposed to film to assure that the previous signal had been completely removed (data not shown). *Arrows* and *X* indicate DNA fragments detected with different probes. The Southern-blot hybridisation is shown in *panel A* and the genetical distance between the probes is shown in panel **B**

than, the average in the potato genome (1 Mb per cM, Tanksley et al. 1992). The distance between CT100 and IPM4 is 0.98 cM in the selfed Cara progeny, from which we conclude from the *Sma*I digest may correspond to 400 kb or less. This indicates that there are about 400 kb/cM in the vicinity of the *Rx* locus, and the approximate distance between the closest flanking markers IPM3 and IPM4 is approximately 100 kb.

Discussion

In this paper we report the high-resolution mapping of the *Rx* locus in the potato cultivar Cara to the top of chromosome XII. The order of the RFLP markers CT99, TG360, CP60, CT100, GP34 and CT129 in the vicinity of *Rx* is consistent with the potato map published previously (Tanksley et al. 1992; Schippers et al. 1994). However, the position of the *Rx* locus in cv Cara relative to GP34 and CP60 is different from the published data for *Rx*1 (Ritter et al. 1991). In Cara the *Rx* locus is centromeric to GP34 and CP60, whereas *Rx*1 was mapped on the telomeric side of these two markers in the resistant line p18 (H82.337/49) (Ritter et al. 1991). One possible explanation of this discrepancy is that *Rx* in Cara is different from the *Rx*1 mapped by Ritter (Ritter et al. 1991). The second possibility could be that there is an inversion at the top of chromosome XII in the line p18 (H82.337/49) relative to the Cara genome. There are precedents consistent with both of these possibilities. The potential for multiple sites of *Rx* is illustrated by the separate *Rx*1 and *Rx*2 loci which, despite being on different chromosomes (Ritter et al. 1991), are functionally identical (Querci et al. 1995). The possibility of a chromosomal inversion in line p18 is illustrated by the genome maps of potato described by Jacobs et al. (1995) showing that the potato genome is prone to chromosomal rearrangements in different cultivars. One of the regions affected by these rearrangement is the arm of chromosome XII carrying *Rx*. To resolve these alternatives it will be necessary to compare the Cara and p18 (H82.337/49) genomes with markers that are closer to *Rx* than the IPM3 and IPM4 markers described here.

Detailed understanding of both the function and the genome organisation of genes conferring extreme resistance against viruses requires the isolation and molecular characterization of *Rx*. Since the biochemical function and the product of *Rx* are not known, we are currently attempting to clone this gene using the closely linked markers described here. The successful outcome of this approach depends on the relationship between genetical and physical distance which may vary by up to 100-fold in different regions of the same genome. For example, in the tomato genome there are 43 kb/cM in the vicinity of the *Fusarium oxysporum I*2 resistance locus on chromosome XII (Segal et al. 1992). By

contrast, in chromosome XI in the region of the *Tm*-2a locus conferring resistance to TMV there are at least 4000 kb/cM (Ganal et al. 1989). We have produced a provisional estimate of the physical and genetical distance relationship around *Rx* in Cara corresponding to 400 kb/cM (Fig. 7). However, an accurate estimate can be generated only by molecular cloning of the DNA around *Rx* using the markers described in this paper. To this end we have developed a library of BAC (bacterial artificial chromosome) clones of DNA from a descendant of Cara carrying *Rx* in the duplex condition. The screening of this library is in progress and will indicate whether the fine-structure genetical map has sufficient resolution for the molecular cloning of *Rx* using the closest flanking markers.

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