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W. De Jong · A. Forsyth · D. Leister
C. Gebhardt · D. C. Baulcombe

A potato hypersensitive resistance gene against potato virus X maps to a resistance gene cluster on chromosome 5

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Abstract The dominant *Nb* gene of potato confers strain-specific hypersensitive resistance against potato virus X (PVX). A population segregating for *Nb* was screened for resistance by inoculating with PVX strain CP2, which is sensitive to *Nb*. Through a combination of bulked segregant analysis and selective restriction fragment amplification, several amplified fragment length polymorphism (AFLP) markers linked to *Nb* were identified. These were cloned and converted into dominant cleaved amplified polymorphic sequence (CAPS) markers. The segregation of these markers in a *Lycopersicon esculentum* × *L. pennellii* mapping population suggested that *Nb* is located on chromosome 5. This was confirmed by examining resistant and susceptible potato individuals with several tomato and potato chromosome-5-specific markers. *Nb* maps to a region of chromosome 5 where several other resistance genes–including *R1*, a resistance gene against *Phytophthora infestans*, *Gpa*, a locus that confers resistance against *Globodera pallida*, and *Rx2*, a gene that confers extreme resistance against PVX–have previously been identified.

Key words *Solanum tuberosum* · Resistance gene cluster · Potato virus X · *Phytophthora infestans* · AFLP

Introduction

Several dominant genes have been identified in potato that confer resistance against potato virus X in a strain-specific fashion. The *Nb* and *Nx* genes each confer hypersensitive resistance against PVX, while *Rx1*, and *Rx2* both confer extreme resistance against this virus (Cockerham 1970; Ritter et al. 1991). The panel of different interactions between PVX and potato resistance (R) genes constitutes an informative system to study the specificity of R gene-virus interactions.

Several PVX strains that differentiate potato R genes have been cloned and sequenced (Huisman et al. 1988; Skryabin et al. 1988; Orman et al 1990; Kavanagh et al 1992; Querci et al. 1993; Feigelstock et al. 1995). Through the construction of hybrids between virulent and avirulent strains, the viral determinants of interaction with *Nx* and *Rx1* have been identified (Santa Cruz and Baulcombe 1993; Goulden et al. 1993; Bendahmane et al. 1995). Both *Nx* and *Rx* appear to "recognize" the viral coat protein, although different features are perceived by each gene.

To more completely understand the basis of specificity in PVX-R gene interactions, it will be necessary to clone and characterize the corresponding R genes. Several R genes have recently been isolated and sequenced from other plants (reviewed in Jones 1996). Although the genes cloned to-date confer resistance against a variety of pathogens, including bacteria, fungi and viruses, clear sequence relationships are evident among these genes. Most of them code for a leucine-rich repeat sequence, an element that may be involved in signal perception, and some express kinase-like domains, possibly involved in signal transduction (Jones 1996). Many of these R genes were isolated by map-based means (Martin et al 1993; Mindrinos et al 1994; Grant et al. 1995; Song et al 1995; Dixon et al. 1996).

Genetic mapping studies have localized *Rx1* to the top arm of chromosome 12 and *Rx2* to the upper arm of chromosome 5 (Ritter et al. 1991). This information provides a useful starting point for the map-based cloning of these genes. Indeed, work is in progress to clone *Rx1* by positional means (A. Bendahmane, K. Kanyuka and D.B., personal communication). Less, however, is known about the chromosomal locations of the

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W. De Jong · A. Forsyth · D. C. Baulcombe (✉)
The Sainsbury Laboratory, Norwich Research Park, Norwich NR4 7UH, UK

D. Leister · C. Gebhardt
Max-Planck-Institut für Züchtungforschung, W-5000 Köln 30, Germany

hypersensitive resistance genes *Nx* or *Nb*. Such information might reveal relationships between these genes and those conferring extreme resistance, or to genes conferring resistance to non-viral pathogens. Map information is also important if these R genes are to be cloned.

To determine where *Nb* is located, we examined progeny which resulted from selfing cv 'Pentland Ivory', a potato variety that carries *Nb*. Through the use of bulked segregant analysis (Michelmore et al. 1991) and AFLP technology (Vos et al. 1995), several markers linked to *Nb* maps to chromosome 5, close to the extreme resistance gene *Rx2*, as well as to *Gpa* (Kreike et al. 1994), a locus that provides resistance against *Globodera pallida*, and *R1* (Leonards-Schippers et al. 1992), a gene that confers hypersensitive resistance to *Phytophthora infestans*.

Materials and methods

Plant and viral materials

The virus-free potato cultivar 'Pentland Ivory' was obtained from the Agriculture and Fisheries Department, East Craigs, Edinburgh. Pentland Ivory carries the *Nb* resistance gene and was selfed to construct a population of 109 progeny segregating for *Nb*.

PVX strain CP, re-named CP2 in this paper to reflect that it is a group-2 strain as defined by Cockerham (1955), is unable to infect potato varieties that carry the *Nb* resistance gene (Moreira et al. 1980). Transcripts from a full-length cDNA alone (S. Santa-Cruz and D.B., unpublished results) of CP2 were synthesized in vitro to provide a genetically uniform source of inoculum, and were used to mechanically inoculate the cotyledons of young tomato seedlings. Two to three weeks after inoculation, virus particles were purified from infected tomato plants. Ten grams of symptom-expressing leaves were homogenized in 20 ml of 0.5 M sodium borate, pH 8.2, and then mixed with 30 ml of chloroform. The phases were separated by a 20-min centrifugation at 3500 g, and the aqueous supernatant was filtered through muslin into new tubes. For each 10 ml of supernatant, 5.25 ml of 20% (w/v) polyethylene glycol (PEG; $M_r = 8000$) was added. The solution was mixed, and left to stand for 1 h at 4°C. The virus was pelleted with a 10-min centrifugation at 13 200 g and then re-suspended in 10 ml of 4% (wv) NaCl. The resulting suspension was centrifuged again for 10 min at 13 200 g and the supernatant was then transferred to a new tube and mixed with 5.25 ml of 20% PEG. After 1 h 4°C, the virus was pelleted as above, re-suspended in 1 ml of 50 mM sodium borate, pH 8.2, and used immediately for potato inoculations.

DNA from F_2 progeny (41 individuals) of an *L. esculentum* × *L. pennellii* mapping cross was kindly supplied by C.M. Thomas (Sainsbury Laboratory, UK). The plants from which the DNA was isolated were originally provided by S. Tanksley (Cornell University, USA). Data for the segregation of over 1000 RFLP markers in these plants is available from the SolGenes Genome Database (http:\\probe.nalusda.gov:8000/cgi-bin/browse/solgenes).

A population of 461 diploid potato progeny segregating for *Phytophthora* resistance conferred by *R1* has previously been described (Meksem et al. 1995), as has a population of 44 diploid progeny segregating for *Rx2* (Ritter et al. 1991).

Screening of the mapping population for resistance against CP2

Stock plants of 109 Pentland Ivory S_1 progeny were maintained in the glasshouse (20°C). Two or more cuttings were taken from each stock plant, allowed to root, and then transferred to a controlled environment chamber (15°C, 85% humidity, 16-h photoperiod, light intensity of 100 $\mu E\,m^{-2}s^{-1}$) at least 1 week prior to inoculation. When the cuttings reached 10–20 cm in height, two leaves from each cutting were mechanically inoculated with CP2 virion preparation. Inoculated leaves were examined 8 days later for the presence of hypersensitive lesions. Three weeks after inoculation, systemic infection of non-inoculated leaves was tested by ELISA, using polyclonal PVX antisera [Agricultural Development and Advisory Service (ADAS), Harpenden] to coat microtitre plates, and monoclonal antibody MAC58 (Torrance et al. 1986) (supplied by ADAS) to detect the virus. Plants were considered resistant if they displayed hypersensitive lesions on inoculated leaves and virus could not be detected in non-inoculated leaves. Plants were considered susceptible if no local lesions were evident and virus could be detected in non-inoculated leaves.

Preparation of genomic DNA for AFLP analyses

To isolate genomic DNA, 5 g of potato leaves were frozen in liquid nitrogen and ground into a fine powder with a mortar and pestle. This powder was transferred to a tube containing 30 ml of extraction buffer [100 mM Tris-Cl, pH 8.0, 50 mM EDTA, 500 mM NaCl, 12.5% SDS, 8.3 mM NaOH, 0.38% w/v) sodium bisulphite, 0.38% (w/v) sodium diethyl-dithio-carbamate] pre-heated to 65°C. After ensuring that all the powder had been wetted, the suspension was placed at 65°C. After ensuring that all the powder had been wetted, the suspension was placed at 65°C for a further 30 min. Potassium acetate (9.3 ml of a 5-M solution) was added to the suspension, and the mixture was then placed on ice for 20 min. Particulate material was pelleted with a 10-min centrifugation at 2600 g and then the supernatant was filtered through muslin. Nucleic acids were precipitated by the addition of 0.7 vol of 2-propanol and pelleted immediately at 2600 g for 10 min. The pellet was washed with 70% ethanol and then dissolved in 2 ml of TE (10 mM Tris-Cl pH 8.0, 1 mM EDTA). An equal volume of CTAB buffer (200 mM Tris-Cl, pH 8.0, 50 mM EDTA, 2 M NaCl, 2% CTAB) was added, and then the solution was mixed and incubated at 65°C for 15 min. The solution was extracted once with chloroform, the aqueous phase was transferred to a new tube, and 0.7 vol of 2-propanol were added. Nucleic acids were again pelleted with a 10-min centrifugation at 2600 g, washed with 70% ethanol, and re-suspended in 4 ml of 25 mM Tris-Cl pH 8.0, 10 mM EDTA. Genomic DNA was then further purified through CsCl density gradient centrifugation (Ausubel et al. 1987). Equivalent amounts of DNA from 20 resistant and 18 susceptible progeny, chosen at random, were bulked to create "resistant" and "susceptible" pools. Both pools were prepared for selective restriction fragment amplification as described by Thomas et al. (1995), using *Mse*I as the 'frequent' cutting enzyme and *Pst*I as the 'rare' cutting enzyme.

AFLP analyses

Selective restriction fragment amplification were performed, and the resulting products separated on 4.5% polyacrylamide gels, exactly as described by Thomas et al. (1995). Briefly, the resistant and susceptible pools were examined with 4 *Pst*I and 24 *Mse*I primers, giving a total of 96 different primer combinations. The *Pst*I primers were complementary to linkers that had been ligated to the *Pst*I sticky ends after digestion; in addition, the *Pst*I primers carried two extra nucleotides as their 3′ ends. Similarly, the *Mse*I primers were complementary to linkers ligated to *Mse*I sticky ends, and carried three extra nucleotides at their 3′ ends. The extra nucleotides at the 3′ ends of the *Pst*I and *Mse*I primers allowed for selective restriction fragment amplification. Side-by-side comparison of the amplified DNA of resistant and susceptible pools allowed easy detection of candidate AFLP markers linked in *cis* to *Nb*.

Cloning of AFLP markers

Restriction fragments amplified specifically from the resistant pool represent sequences that may be linked in *cis* to *Nb*. Slices containing such fragments were excised from 4.5% acrylamide gels that had previously been dried on Whatmann 3 MM paper. The gel slices were re-hydrated in 1 ml of TE for 1 h at room temperature. The slices were then transferred to new tubes containing 200 µl of TE, crushed with a pipette tip, and left for another 2 h. After soaking, the crushed slice was pelleted with a brief centrifugation at 12 000 g. One microliter of supernatant was then used as a template in a further polymerase chain reaction (PCR) amplification, using the same reaction conditions and the same pair of primers that had originally been used to amplify the restriction fragment from the resistant pool. These PCR products were than cloned directly into the vector pGEM-T (Promega). To confirm that a clone corresponded to the desired fragment, each clone was used as a template for a further amplification, using the same *Pst*I and *Mse*I primers and reaction conditions that had revealed the initial AFLP.

RFLP markers

The tomato chromosome-5 RFLP markers CT167 and TG432 (Tanksley et al. 1992) were kindly provided by S. Tanksley (Cornell University, USA). Map locations of potato chromosome-5 RFLP markers GP21, GP179 and GP186 (Leonards-Schippers et al. 1992) and conversions of GP21 and GP179 into PCR markers have previously been described (Meksem et al. 1995).

Conversion of AFLP and RFLP markers into cleaved amplified polymorphic sequence (CAPS) markers

The ends of AFLP and RFLP markers were sequenced with M13 forward and reverse primers. For all RFLP markers and large (>200 bp) AFLP markers, this sequence information was used to design oligonucleotide primers for direct PCR amplification of the RFLP or AFLP locus. For small AFLP markers (<200 bp), primers were first designed for a cycle of inverse PCR (I-PCR). Following the I-PCR protocol of Thomas et al. (1994), sequences flanking the small AFLP markers were cloned and sequenced, and this information was then used to design primers for direct PCR amplification of the AFLP locus. Undigested DNA from resistant and susceptible pools was amplified with forward and reverse primer pairs corresponding to each RFLP or AFLP locus. In all cases, amplification of resistant and susceptible pools resulted in products of identical size. To search for polymorphism between the PCR products of resistant and susceptible pools, the products of each were digested with a panel of restriction enzymes with four-base recognition specificities. If a polymorphism was detected after digestion, it was confirmed by examining individual members of the resistant and susceptible pools.

PCR-based screening of the mapping population

Initially, 109 Pentland Ivory S_1 progeny were screened for resistance and susceptibility. Some of the stock plants died or did not tuberise, leaving only 90 plants (68 resistant and 22 susceptible) for subsequent analysis. Template DNA for CAPS analysis (Konieczny and Ausubel 1993) was prepared from the 90 remaining plants by grinding a small leaf disc of each plant in a 1.5-ml tube containing 200 µl of 0.14 M sorbitol, 0.022 M EDTA, 0.8 M NaCl, 0.8% CTAB, 1% sodium n-lauroylsarcosine and 0.22 M Tris-Cl, pH 8. Chloroform (200 µl) was then added to each tube, the contents were mixed, and the emulsions were incubated at 65°C for 5 min. The tubes were centrifuged at 12 000 g for 5 min, and then 100 µl of supernatant were transferred to a new tube containing 100 µl of isopropanol. DNA was allowed to precipitate for 15 min at room temperature, and then collected by a 15-min centrifugation at 12 000 g. The resulting pellet was washed with 70% ethanol, air dried, and resuspended in 50 µl of water. Two microliters of this DNA were used as a template in amplification reactions. All CAPS analyses of individuals in the mapping population were conducted in 50-µl reaction volumes containing 10 mM Tris-Cl pH 8.3, 0.4 mM dNTPs, 2.5 mM $MgCl_2$, 50 mM KCl, 0.05% nonidet-P40 and 0.5 µM of each oligonucleotide primer. Primer sequences and details of thermal cycling conditions for each CAPS marker are given in Table 1.

Construction of map around *Nb* locus

To analyse the data from tetraploid Pentland Ivory S_1 progeny, we used the program MapMaker 2.0 (Lander et al. 1987), and assumed a negligible frequency of double reduction. Without double reduction, 50% of gametes from a simplex parent will carry *Nb*, which is the same proportion of gametes that would carry *Nb* if a heterozygous diploid parent had been used. Thus, the data were treated as if they had arisen from a diploid self (*Nbnb* × *Nbnb*).

Results

Development of a segregating population

The tetraploid potato cultivar 'Pentland Ivory' carries the *Nb* resistance gene. To construct a population segregating for *Nb*, Pentland Ivory was selfed. Two or

Table 1 PCR markers linked to *Nb*

Marker name	Forward and reverse primer sequences (5′ to 3′)	Cycling conditions (35 cycles)
GP186	TTCTATTACTCATGAAGGCTCTCC AATCATAATCACGACTCATTGTCC	94°C, 15 s; 55°C, 15 s; 72°C, 90 s
SPUD839	GACATGAGTTTTAGCAACAGTG AAGTAGCTGATATTGGGGATTC	94°C, 15 s; 55°C, 15 s; 72°C, 40 s
GP21	GGTTGGTGGCCTATTAGCCATGC AGTGAGCCAGCATAGCATTACTTG	94°C, 15 s; 60°C, 15 s; 72°C, 60 s
TG432	AAAGCAGTTGGATCTTCATCATCTC CGTTCAACACTGGTGCAATGAGAAG	94°C, 15 s; 55°C, 15 s; 72°C, 120 s
SPUD237	TTCCTGCTGATACTGACTAGAAAACC AGCCAAGGAAAAGCTAGCATCCAAG	94°C, 15 s; 55°C, 15 s; 72°C, 40 s
SPUD128	GTCAGCTCACGAATTCACATTCC GTGCAGACTCTGCCTAATCGACTC	94°C, 15 s; 50°C, 15 s; 72°C, 40 s

more cuttings from each of the S_1 progeny were screened for resistance to PVX by mechanically inoculating two leaflets of each cutting with strain CP2, which is sensitive to the *Nb* gene. By 8-days post-inoculation, 84 of the S_1 progeny had developed distinctive hypersensitive lesions on the inoculated leaves, characteristic of the *Nb*-mediated resistance response. The remaining 25 S_1 progeny did not develop lesions. Three weeks after inoculation, PVX could readily be detected by DAS-ELISA in the non-inoculated leaves of the 25 progeny which had not developed lesions, indicating that these plants were susceptible to PVX strain CP2. The observed 84 : 25 segregation ratio is a good fit to the 3 : 1 segregation ratio predicted if Pentland Ivory carries *Nb* in the simplex condition (i.e. genotype *Nbnbnbnb*). In a few cases, virus could be detected at levels comparable to susceptible plants in the non-inoculated leaves of plants which had developed local lesions. However, this was not reproducible for independent cuttings of any clone, and was always associated with the presence of hypersensitive lesions in the non-inoculated leaves, suggesting that PVX could occasionally escape the plant defences in the inoculated leaves, only to trigger the hypersensitive response again in the non-inoculated leaves.

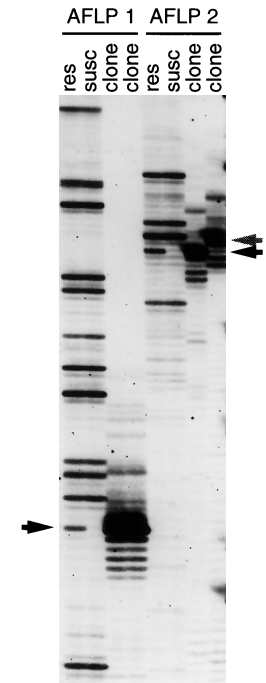

Fig. 1 Examples of two AFLP markers linked to *Nb*. Selected restriction fragments were amplified from pools of DNA from resistant (*res*) and susceptible (*susc*) S_1 progeny. *Black arrows* denote AFLPs, i.e. fragments specifically amplified from the resistant pool. Putative clones of each AFLP were amplified with the same primer combination to assess insert sizes. The *uppermost (grey) arrow* indicates an insert of inappropriate size.

Identification of markers linked to *Nb*

To identify markers linked to *Nb*, DNA from resistant and susceptible progeny was bulked to create resistant and susceptible pools. Each pool was then examined with 96 primer combinations, to identify AFLP markers linked to *Nb*. The primer combination each amplified approximately 100 fragments, so that about 9600 fragments were screened for linkage to *Nb*. Of the 96 primer combinations, seven revealed a total of eight fragments specific to the resistant pool. The AFLPs ranged in size from 40 to 400 bp; two representative AFLPs are shown in Fig. 1.

It is our long term goal to clone *Nb* by using closely linked DNA markers. In addition to identifying many AFLP markers linked to *Nb*, this process will require the identification of chromosomal recombination events near the *Nb* locus. To facilitate the screening of progeny for recombination events near *Nb*, several AFLP markers were cloned, and then converted into CAPS markers (Koniecany and Ausubel 1993).

To clone the AFLP markers the DNA was eluted from dried-down gels and re-amplified using the same primer combination that had originally been used to identify each polymorphic fragment. The resulting PCR products were then ligated into a plasmid vector. To assess whether the cloned fragments were of the same size as the initial AFLPs, the DNA of individual clones was amplified with the primer combination used to generate each AFLP, and the ensuing PCR products were compared with the original AFLP on a sequencing gel (Fig. 1). While most clones tested had inserts of the correct size, some did not (Fig. 1).

Cloned DNA corresponding to the size of the AFLP was sequenced. Based on the sequence, primers were designed for direct amplification of the corresponding locus if the AFLP was greater than 200 bp in length. Alternatively, if the AFLP was smaller than 200 bp in length, primers were designed for an intermediate step of inverse PCR (I-PCR). When I-PCR was employed, the I-PCR products were cloned and sequenced. Based on this sequence-data further primers were designed for direct amplification of the AFLP and its surrounding DNA.

After cloning and sequencing an AFLP marker it is not possible, without cloning and sequencing all alternative alleles, to determine what sequence polymorphism gave rise to the AFLP. Thus additional polymorphisms linked to resistance were sought by digesting the PCR products corresponding to each AFLP locus with a panel of restriction enzymes. For three of six cloned AFLP markers, this strategy led to the identification of DNA polymorphism linked to *Nb*. The CAPS marker SPUD237, illustrated in Fig. 2, was identified in this way, as were CAPS markers SPUD839 and SPUD128 (gel images not shown; primer sequences are given in Table 1).

Analysis of interspecific tomato cross suggests a chromosome-5 location

The genomes of potato and tomato show a remarkable conservation of gene order (Bonierbale et al. 1988;

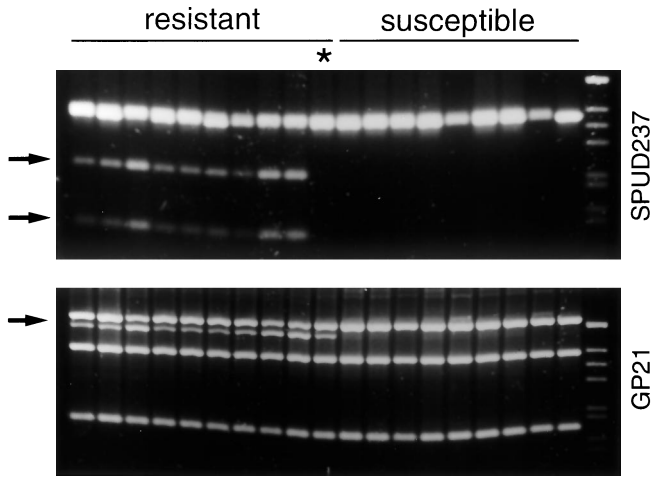

Fig. 2 Examples of two CAPS markers linked to *Nb*. DNA from ten resistant and nine susceptible S_1 progeny was PCR-amplified with SPUD237 forward and reverse primers, or GP21 forward and reverse primers (Table 1). After amplification, all PCR products were digested with *Alu*I, separated on a 2% agarose gel, and visualized by staining with ethidium bromide. *Arrows* denote digestion products that are linked to resistance. The resistant plant marked with an *asterisk* carries a recombination event between *Nb* and SPUD237; this was the only recombinant identified between *Nb* and SPUD237 in a mapping population of 90 S_1 progeny

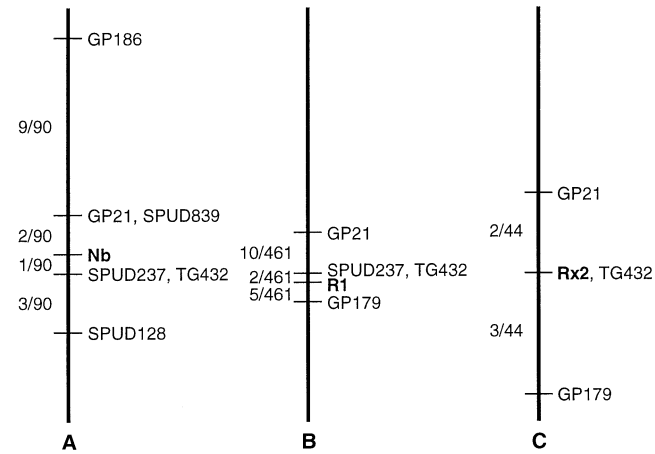

Fig. 3A–C Comparative map locations of *Nb*, *R1*, and *Rx2*. Genetic maps shown were obtained by mapping common markers in three different segregating populations (see Materials and methods). **A** Marker order around *Nb*. **B** Marker order around *R1*. **C** Marker order around *Rx2*. All markers shown are PCR-based. The SPUD markers were derived from AFLP markers linked to *Nb*, while GP21, GP179, GP186 and TG432 were derived from chromosome 5-specific RFLP markers of the same name. The number of recombination events/number of plants scored is shown to the left of each marker interval

Gebhardt et al. 1991). One high-density genetic map for solanaceous plants was obtained by scoring over 1000 RFLP markers in the F_2 progeny of an interspecific tomato cross (*L. esculentum* × *L. pennellii*; Tanksley et al. 1992). We found that two CAPS markers linked to *Nb*, SPUD237 and SPUD128, revealed a polymorphism between *L. esculentum* and *L. pennellii* (data not shown). The segregation of these markers in the tomato mapping population suggested a chromosome-5 location for *Nb*, as both SPUD237 and SPUD128 mapped to the upper arm of tomato chromosome 5, within 10 cM of tomato RFLP markers CT167 and TG432. This location was confirmed by RFLP analysis in potato with CT167 (data not shown) and was further confirmed by the conversion of potato and tomato chromosome-5 RFLP markers into CAPS markers that co-segregated with *Nb*. The co-segregation of *Nb* with CAPS marker GP21 is illustrated in Fig. 2. Similar data were obtained with tomato chromosome-5 marker TG432 and potato chromosome-5 marker GP186 (data not shown).

Construction of a genetic map around *Nb*

The segregation of six CAPS markers linked to *Nb* was monitored in a mapping population of 90 Pentland Ivory S_1 progeny. The data were analysed with MapMaker (Lander et al. 1987) and are summarized in Fig. 3 A. As the tomato data suggested, *Nb* maps to the upper arm of potato chromosome 5. *Nb* resides in an approximately 3-cM interval defined on the telomeric side by co-segregating markers GP21 and SPUD839 and, on the centromeric side, by co-segregating markers TG432 and SPUD237.

Nb is located in a resistance gene cluster

Three other resistance genes have previously been mapped to this region of chromosome 5. *R1*, a gene that confers hypersensitive race-specific resistance to *P. infestans*, was mapped 2.5 cM to the centromeric side of RFLP marker GP21 (Leonards-Schippers et al. 1992). *Rx2*, a gene that confers extreme resistance to most strains of PVX, was mapped 4.5 cm centromeric of GP21 (Ritter et al. 1991). *Gpa*, a locus that confers resistance to *G. pallida*, also maps to this area (Kreike et al. 1994). To further examine the relationship between *Nb* and *R1*, a population of 461 individuals segregating for *R1* was examined with the markers SPUD237 and TG432. *R1* was found to map just slightly centromeric to both of these markers (Fig. 3 B), confirming that *Nb* and *R1* are closely linked, and also demonstrating that *R1* and *Nb* are not allelic. In a smaller mapping population of 44 individuals segregating for *Rx2*, no recombinants were found between TG432 and *Rx2* (Fig. 3 C), showing that *Nb* and *Rx2* are also closely linked. Although *Nb*, *Rx2* and *R1* have all been mapped in different populations it is likely that these loci occupy linked positions in the potato genome.

Discussion

The potato resistance gene *Nb* maps to the upper arm of chromosome 5 in a region where several other resistance genes-*R1*, *Gpa*, *Rx2*-have previously been located. In addition to other examples in potato (Leister et al. 1996), clustering of disease resistance genes has also been observed in maize (McMullen and Simcox 1995), lettuce (Kesseli et al. 1993), and soybean (Kanazin et al, 1996; Yu et al. 1996). Clustering may reflect localized resistance gene duplication and subsequent divergence events, since the resistance genes sequenced to-date fall into a small number of related classes (reviewed in Jones 1996). Functionally, the clustering of resistance genes may facilitate coordination of defence against diverse pathogens. Further high-resolution characterization of chromosome 5 in the region of *Nb*, *R1, Gpa* and *Rx2* should provide useful insight into resistance gene organization and function.

It is particularly intriguing that *Nb*, a gene that confers hypersensitive resistance against some strains of PVX, maps so closely to *Rx2*, a gene that confers extreme resistance to most strains of the same virus. Our data do not exclude the possibility that *Nb* and *Rx2* are alleles, although higher-resolution map data would be needed to conclude this with any confidence. *Rx1* is known to "recognize" a feature of the viral coat protein (Goulden et al. 1993; Bendahmane et al. 1995), and *Rx2* apparently recognizes the same feature (Querci et al. 1995). The avirulence determinant of the *Nb*-PVX interaction has yet to be fully characterized, but it is not the PVX coat protein (W.D., A.F. and D.B., unpublished results).

Nb and the extreme PVX resistance gene X^i have previously been reported to be unlinked (Solomon 1985). This result apparently contradicts the finding reported here that *Nb* and *Rx2* are linked because it has been assumed that X^i and *Rx2* are homonyms. However, the nomenclature for the extreme resistance genes against PVX varies, and the relationships among the various genes is not always clear. In view of the close linkage between *Nb* and *Rx2*, it seems most likely that X^i is synonymous with *Rx1*, or conceivably a third *Rx* locus. The *Nb* gene studied by Solomon (1985) is very likely the same gene studied here, as marker SPUD237 is linked to both copies of the *Nb* gene present in cultivar 'Catriona' (W.D., A.F. and D.B., unpublished results), which was the source of *Nb* in the Solomon (1985) study.

The mapping of *Nb* was initiated with the long-term view of cloning this gene by "chromosome landing" (Tanksley et al. 1995). To generate a high-resolution map around the *Nb* locus will require both a large number of markers lined to *Nb* and a large number of recombination events around *Nb* to determine the relative order of all closely linked markers. With the advent of AFLP methodology (Vos et al. 1995) it is relatively straightforward to generate a large number of markers. Using just 96 primer combinations we identified eight markers linked to *Nb*. Several hundred more primer combinations are available, and so it should be possible to identify many more markers. The CAPS markers SPUD237 and GP21 (Fig. 2) flank *Nb*, and will be useful in identifying recombination events close to *Nb*. Some 700 further plants have already been screened with these markers, identifying additional recombinants in this interval (W.D and D.B., unpublished results) and providing further evidence that *Nb* maps between GP21 and SPUD237, rather than outside of this interval.

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