Tight Physical Linkage of the Nematode Resistance Gene *Gpa2* and the Virus Resistance Gene *Rx* on a Single Segment Introgressed from the Wild Species *Solanum tuberosum* subsp. *andigena* CPC 1673 into Cultivated Potato

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Comparative mapping studies within several plant genera have shown that genes for resistance to taxonomically diverse pathogens are often co-localized on the maps of these genotypes. Here we describe the genetic analysis of such a gene cluster on chromosome 12 of potato. This cluster harbors the resistance loci *Gpa2* and *Rx*, which confer resistance to the potato cyst nematode *Globodera pallida* and potato virus X (PVX), respectively. By screening two mapping populations with 10 CAPS (cleaved amplified polymorphic sequence) markers derived from AFLP (amplified fragment length polymorphism) markers and the ends of overlapping bacterial artificial chromosome (BAC) clones, it is demonstrated that *Gpa2* and *Rx* are tightly linked. From a recombination fraction of 1/3386, found after screening two different mapping populations, a genetic distance of 0.02 cM between *Gpa2* and *Rx* was calculated. The physical length of this genetic interval does not exceed 300 kb of DNA as determined by the total size of overlapping BAC clones in the *Rx/Gpa2* contig. Evidence is provided that both genes have been introgressed from a single wild species clone, *Solanum tuberosum* subsp. *andigena* CPC 1673. This suggests that these genes underlying the disease resistance specificities to potato virus X and potato cyst nematodes have a common evolutionary origin.

Most plant resistance genes (*R* genes) are dominantly inherited and are often involved in resistance processes that are

characterized by a hypersensitive response (HR; Staskawicz et al. 1995; Crute and Pink 1996; Hammond-Kosack and Jones 1996). Two distinct genetic arrangements have been found among *R* loci: either single loci, many different alleles of which can occur in the germplasm (Bent et al. 1994; Mindrinos et al. 1994; Lawrence et al. 1995; Grant et al. 1995), or different genes that occur at compound loci forming *R*-gene clusters (Jones et al. 1994; Martin et al. 1993, 1994; Dixon et al. 1996; Anderson et al. 1997; Parniske et al. 1997; Song et al. 1997). The occurrence of different *R* genes on the same genomic location in different species of a plant genus has been ascribed to the common origin of *R* gene families (Baker et al. 1997). This is exemplified by the recent characterization of the *Cf-4/9* locus of tomato. The *R* genes *Cf-4* and *Cf-9* originate from the wild species *Lycopersicon hirsutum* and *L. pimpinellifolium*, respectively, and confer resistance to different races of the fungal pathogen *Cladosporium fulvum*. Sequencing of the entire region in both the wild and the cultivated species revealed several (active) *R* gene homologs, indicating that frequent sequence exchange between these tandemly repeated genes created novel *R* gene specificities (Thomas et al. 1997; Parniske et al. 1997). Similar evolutionary processes and subsequent diversification in different plant species may also underlie the generation of novel disease resistance specificities against different pathogen species. For example, a complex locus is known on chromosome 5 of potato. This cluster comprises at least five *R* loci: *RI*, which bestows resistance to *Phytophthora infestans* (Leonards-Schippers et al. 1992); *Nb*, conferring an HR type of resistance, and *Rx2*, conferring extreme resistance to potato virus X (De Jong et al. 1997; Ritter et al. 1991); and *Gpa* and *Grp1*, conferring resistance to the potato cyst nematode (Kreike et al. 1994; Rouppe van der Voort et al. 1998). All these loci originate from different genetic backgrounds.

The occurrence of *R*-gene-like sequences at compound *R* loci has recently been demonstrated for a number of plant spe-

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cies (Martin et al. 1993, 1994; Dixon et al. 1996; Leister et al. 1996; Anderson et al. 1997; Parniske et al. 1997; Song et al. 1997). However, it is impossible to predict from the DNA sequence whether these genes are functional to different pathogen species. At present, only a few examples exist that shed light on the physical linkage of *R* genes mapped at complex loci that respond to unrelated (pathogen) signals. The genes encoding Pto and Fen in tomato, which induce hypersensitive-like necrosis upon recognition of the bacterial avirulence gene product AvrPto and the chemical compound Fenthion, respectively (Martin et al. 1994; Tang et al. 1996; Scofield et al. 1996), are localized several tens of kilobases from each other (Salmeron et al. 1996; Rommens et al. 1995). In lettuce, the *Dm6* locus to downy mildew and the root aphid resistance locus *Ra* are genetically linked and originate from the same *Lactuca serriola* accession (Crute and Dunn 1980; Crute 1992). However, from the size of the genetic interval, these loci may still lie 6 Mb apart (Anderson et al. 1996). A remarkable case is the *Mi* gene in tomato. This gene exhibits a pleiotrophic effect and confers resistance to different species of the root knot nematode (*Meloidogyne* spp.) and the potato aphid *Macrosiphum euphorbiae* (Milligan et al. 1998; Rossi et al. 1998).

Here, the identification of a novel *R* gene cluster in potato is described. *Gpa2*, which bestows specific resistance to a small set of populations of the potato cyst nematode *Globodera pallida* (Roupe van der Voort et al. 1997) and *Rx*, which confers extreme resistance to potato virus X (PVX; Ritter et al. 1991; Bendahmane et al. 1997), were previously mapped on the short arm of chromosome 12. We now provide evidence that both genes are physically tightly linked and that they reside on an introgression segment that was introduced into a number of European potato cultivars from a single wild potato clone, *Solanum tuberosum* subsp. *andigena* CPC 1673.

RESULTS

Cosegregation of *Gpa2* and *Rx* resistance.

The resistance loci *Gpa2* and *Rx* were previously mapped in separate mapping populations to the same 6 cM genetic interval on the distal end of chromosome 12, between the RFLP (restriction fragment length polymorphism) markers GP34 and CT79 (Roupe van der Voort et al. 1997; Bendahmane et al. 1997). Linkage of *Gpa2* and *Rx* was assumed because the resistance donors of both mapping populations, i.e., the diploid genotype SH83 and the tetraploid cv. Cara, harbor introgressions from the same wild potato clone, *Solanum tuberosum* subsp. *andigena* CPC 1673. To determine the degree of linkage between both genes, segregation of *Gpa2* and *Rx* was followed in a selected set of progeny of population F1SH×RH and S1-Cara.

Gpa2 and *Rx* resistance tests were performed on the parental genotypes Cara, SH83, and RH89, four S₁ genotypes that are recombined in a 1.21 cM interval between markers GP34 and IPM5 (Bendahmane et al. 1997), and two F₁ genotypes that harbor cross-over events in a 6 cM interval between markers GP34 and CT79 (Roupe van der Voort et al. 1997). These progeny clones exhibit the closest recombination events to *Gpa2* and *Rx* and were previously used to border the *Gpa2* and *Rx* intervals. The results of the resistance tests showed a clear correlation between the number of cysts of *G. pallida*

population Pa₂-D383 able to develop on the roots of the tested genotypes and resistance to PVX_{CP4} (Fig. 1). On the basis of the *G. pallida* Pa₂-D383 test the S1-Cara and F1SH×RH genotypes could be assigned to a resistant class (mean number of cysts 37 ± 7 [SD]) or a susceptible class (mean 715 ± 62 [SD]). These plants showed either absence or presence of a hybridization signal on dot blots of total RNA of the same plants infected with PVX_{CP4}. Resistance tests with *G. pallida* population Pa₃-Rook (results not presented) showed that cv. Cara is susceptible to this nematode population, indicating the specificity of the *G. pallida* resistance. The results of the Pa₂-D383 and PVX_{CP4} tests on this selected set of plant genotypes indicated that *Gpa2* and *Rx* cosegregate in both mapping populations with a maximum probability of $P = 0.016 (= (1/2)^6)$ that the observed linkage could be explained by chance. The S1-Cara recombinants were previously used to localize *Rx* in a 1.21 cM interval between markers IPM3 and IPM5 (Bendahmane et al. 1997). Cosegregation of *Gpa2* with *Rx* suggested that *Gpa2* also resides in this region (Fig. 2A).

Fine mapping of *Gpa2*.

Fine mapping of the *Gpa2* locus was carried out with cleaved amplified polymorphic sequence (CAPS; Konieczny and Ausubel 1993) markers that were originally developed for the fine mapping of the *Rx* locus (Bendahmane et al. 1997; Kanyuka et al., *in press*). These CAPS markers are derived from the *Rx* flanking amplified fragment length polymorphism (AFLP) markers PM3, PM4, and PM5 (Bendahmane et al. 1997) and from the end sequences of BAC (bacterial artificial chromosome) clones, previously isolated from a BAC library prepared from cv. Cara, that map to the GP34-IPM5 interval (Kanyuka et al., *in press*). In addition, two other CAPS markers, IPM4a and IPM4c, were developed via a low-stringency PCR (polymerase chain reaction) screening assay (Kanyuka et al., *in press*). CAPS marker IPM4 was designated IPM4a as digestion of low-stringency IPM4 products identified not only the original IPM4 locus but also new DNA fragments that are polymorphic between PVX-resistant and PVX-susceptible genotypes (Kanyuka et al., *in press*). One of these marker alleles was designated IPM4c. The relative positions of the CAPS markers are depicted in Figure 2B.

To identify recombination events in the IPM3-IPM5 region, a total of 2,788 S1-Cara genotypes were screened for the presence of markers IPM3 and IPM5. In addition, 598 genotypes from population F1SH×RH were subjected to a GP34-IPM5 marker screening as marker IPM3 is not informative in this population. This resulted in the identification of 20 IPM3-IPM5 recombinants in the S1-Cara population and 9 GP34-IPM5 recombinants in the F1SH×RH population. To map these recombination events more precisely, a second marker screening was carried out with all the markers available in the IPM3-IPM5 region (Figs. 2B and 3). Subsequent *G. pallida* Pa₂-D383 tests on these genotypes showed that *Gpa2* is localized between markers IPM4c and 111R. Interestingly, among the 2,788 S1-Cara genotypes and 598 F1SH×RH genotypes tested, only one genotype, S1-761, was identified in which a recombination event had occurred between *Gpa2* and *Rx*. On the other side of *Gpa2*, genotype S1-B811 was used to identify marker 111R as a flanking marker for the *Gpa2* interval. The marker order deduced from the analysis of the F1SH×RH population corresponds to that found in population

S1-Cara. Therefore, estimates of recombination frequencies deduced from both populations were merged to obtain an estimate of the average recombination value for each marker interval (Table 1). The graphical genotypes (Young and Tanksley 1989) shown in Figure 2B display the boundaries of the *Rx* and *Gpa2* intervals. Since there is only one recombinant found between *Gpa2* and *Rx* the average genetic distance between *Gpa2* and *Rx* is estimated to be 0.02 cM.

Physical linkage of *Rx* and *Gpa2*.

The preparation of a BAC library from cv. Cara and the subsequent identification and isolation of Cara BAC clones that map to the IPM3-IPM5 *Rx* interval has been described in Kanyuka et al. (*in press*). Figure 2C depicts the relative positions of four previously identified Cara BAC clones (BAC77, BAC45, BAC221, and BAC111) that map to the 0.04 cM IPM4c-111R genetic interval harboring the *Gpa2* locus. As these BAC clones do not fully cover the *Gpa2* locus and additional PCR screening of the Cara BAC library with CAPS markers 45L and 77R (see Figure 2C) failed to yield a Cara BAC clone that spanned the remaining gap between BAC77 and BAC45, a second BAC library was prepared from the diploid

potato genotype SH83. This library consists of 60,000 clones with an average insert size of 100 kb (data not shown), representing between six and seven haploid genome equivalents of potato. PCR screening of this SH83 BAC library with markers 45L and 77R yielded a BAC clone of 110 kb (SHBAC43) that spans the gap between BAC77 and BAC45, resulting in a contiguous physical map of the *Gpa2* locus (Fig. 2C).

Previously, it was shown that BAC77 spans the *Rx* gene (Kanyuka et al., *in press*). By adding the separate insert sizes of the individual BAC clones in the *Rx/Gpa2* region (Fig. 2C), the maximum physical size of the *Rx/Gpa2* interval was determined to be 300 kb. This figure combined with the calculated genetic distance of 0.04 cM for the interval between markers 77L and 111R results in a ratio of 7.5 Mb/cM for this introgression segment.

Origin of *Gpa2* and *Rx* resistance.

To address the question of whether *Gpa2* and *Rx* were jointly introgressed from the same wild species clone, a subset of the progeny of S1-Cara and F1SH×RH as well as two potato clones derived from a selfing of the original wild clone (abbreviated as CPC 1673-a and CPC 1673-b) and nine potato

Fig. 1. Cosegregation of *Gpa2* and *Rx* resistance demonstrated on the parental clones Cara, SH83, and RH89 as well as on four offspring genotypes of population S1-Cara (S1-152, S1-421, S1-160, S1-1189) and two genotypes of population F1SH×RH (F1-27, F1-140). **A**, Cysts recovered from the roots of one replicate of each plant. Magnification ×5. **B**, Hybridization signal on dot blots of total RNA of the same set of plants infected with PVX_{CP4}.

cultivars harboring introgressions from clone *Solanum tuberosum* subsp. *andigena* CPC 1673 (hereinafter referred to as CPC 1673) were evaluated for the presence of common DNA marker alleles in the GP34-IPM5 region of chromosome 12. As the primers for these CAPS markers may amplify various sequences scattered all over the genome, only the DNA fragments linked in coupling phase to *Rx* were scored.

The CAPS marker profiles of the analyzed potato genotypes harboring introgressions from CPC 1673 were highly similar to those of the selfed CPC 1673 genotypes. All CAPS marker alleles in coupling phase to *Rx* appeared to be of CPC 1673 origin (Fig. 3; Table 2). Among the seven CPC 1673 cultivars tested, a remarkably high number of differences in the size of introgressed region of 0.9 cM were

Fig. 2. **A**, Relative position of the *Rx/Gpa2* interval on potato chromosome 12. **B**, Graphical genotypes showing differences in the size of the CPC1673-derived segments. Presented genotypes border respective intervals of *Gpa2* and *Rx*. Introgression segments indicated by thick bars. Marker interval size in **B** is not on scale. **C**, Physical map of the *Rx/Gpa2* region. Open rectangles represent bacterial artificial chromosome (BAC) clones derived from the S1-781 BAC library. Gray shaded rectangle indicates BAC clone SHBAC43 isolated from the BAC library of genotype SH83. Estimated sizes of the BAC clones are given between parentheses.

observed. A total of five different haplotypes of the CPC 1673-derived chromosomal segment was identified. These differences in introgression segment lengths led unambiguously to the same marker order. Hence, it is concluded that *Gpa2* and *Rx* originate from the same wild species clone and were simultaneously introgressed into domesticated potato cultivars.

DISCUSSION

The hypothesis that both *Gpa2* and *Rx* may have originated from *S. tuberosum* subsp. *andigena* CPC 1673 was tested by screening two different mapping populations for nematode and virus resistance. This screening was aided by a wealth of DNA markers available in the *Rx* region (Bendahmane et al.

Fig. 3. CAPS (cleaved amplified polymorphic sequence) marker analysis on a set of S1-Cara and F1SH×RH progeny as well as a series of *Solanum tuberosum* subsp. *andigena* CPC 1673-derived potato clones. **A**, Marker 77L bracketing left border of the *Rx* interval by clone SH83. **B**, Marker IPM4c bracketing right border of *Rx* interval and left border of *Gpa2* interval. **C**, Marker 77R bracketing left border of *Gpa2* interval. See also Figure 2.

1997; Kanyuka et al., *in press*). Evidence was found that *Gpa2* and *Rx* indeed derive from the same wild species clone since all the marker alleles that flank *Gpa2* and *Rx* were identified in selfings of the original CPC 1673 clone. In addition, these marker alleles also occurred in the Pa₂-D383 and PVX_{CP4} resistant cultivars that harbor introgressions from CPC 1673.

Tight genetic linkage of both genes was demonstrated after segregation analysis in 3,386 offspring derived from two populations. For the IPM3-IPM5 region, 20 recombinant genotypes were identified, of which only one clone showed recombination between *Gpa2* and *Rx*. Compared with this recombination fraction, the number of introgression segment length differences among the CPC 1673 cultivars tested was remarkably high. The CPC 1673 cultivars tested lacked different parts of the IPM3-IPM5 region despite the fact that four out of five of these clones were probably selected for PVX resistance. This may be explained by the relatively early use of *S. tuberosum* subsp. *andigena* CPC 1673 in breeding programs that aimed to introgress resistance into cultivated *Solanum* spp. Selection based on agronomically important traits originating from *S. tuberosum* may have severely reduced the fraction of CPC 1673-derived DNA in concurrent hybrids. This explanation may also be applicable to the high level of allelic variation found at an RFLP locus near the *H1* gene (Niewöhner et al. 1995). From this study, it appeared that only four out of 87 potato clones, known to be resistant to the Ro₁ pathotype of the potato cyst nematode *G. rostochiensis*, harbor a molecular marker allele that is tightly linked to the resistance locus *H1*. It is noted that the recombination events in the IPM3-IPM5 interval occurred after introgression into cultivated potato because the cultivars that had the same selfed

CPC 1673 clone in their pedigree (e.g., cvs. Multa and Saturna from CPC 1673-1 and Alcmaria and Amaryl from CPC 1673-20) contained introgression segments of different lengths. The finding that a considerable level of variation exists between the sizes of the introgressed segments provides an additional source of “rare recombinants” within a selected region of wild origin.

Significant differences in recombination frequencies were observed between marker intervals GP34-77L and 73L-218R and the intervals in the region 77L-73L. Given the physical sizes of the intervals GP34-IPM3 (110 kb; Kanyuka et al., *in press*), 77L-111R (<300 kb), and 73L-IPM5 (about 150 kb; Kanyuka et al., *in press*) it is concluded that the recombination frequency in the 77L-111R interval is significantly reduced. The reduced recombination frequency in the 77L-111R interval, a region that is of wild species origin, may be the result of a high level of sequence heterogeneity between the homologous chromosomes within this interval. This assumption is based on the model that sequence homology between two DNA strands initiates reciprocal genetic exchange during meiosis I (Borts and Haber 1987; Priebe et al. 1994). As a result, a reduction in the recombination frequency will be encountered in regions of heterogeneous origin (containing introgressions of foreign DNA), compared with regions that have a common origin. The suppressive effect of introgressions on recombination frequencies has been shown, e.g., in barley (Görg et al. 1993) and in tomato (Rick 1969; Liharska et al. 1996).

The identification of two *R* genes that originate from a single wild species clone is very fortunate since the original *S. tuberosum* subsp. *andigena* CPC 1673 clone has been selected

Table 1. Number of recombinants, average recombination frequency, and standard error per marker interval

No. of recombinants per cross	GP34-77L	77L-IPM4c	IPM4c-77R	77R-IPM4a	IPM4a-111R	111R-73L	73L-218R	218R-IPM5
Recombinants in S1-Cara (no.)	10 + 6 ^z	0	1	0	1	3	7	2
Recombinants in FISH×RH (no.)	5	0	0	0	0	0	4	0
Recombination (%)	0.545 a	0 b	0.02 b	0 b	0.02 b	0.055 b	0.24 c	0.035 b
± standard error	± 1.2		± 0.95		± 0.95	± 0.95	± 1.0	± 0.95

^z Ten recombinants were found among 1,720 genotypes tested in the interval GP34-IPM3 (Bendahmane et al. 1997). Six recombinants were identified in the interval IPM3-77L among the 2,788 genotypes of S1-Cara. Recombination frequencies followed by a different letter differ significantly in a χ^2 test at $P < 0.05$.

Table 2. Potato clones with *Solanum tuberosum* subsp. *andigena* CPC1673 in their pedigree (with the exception of clone RH89) tested on the presence of chromosome 12 specific CAPS alleles^v

Clone	<i>Gpa2</i>	<i>Rx</i>	IPM3	191L	77L	IPM4c	77R	IPM4a	111R	73L	218R	IPM5
CPC1673-a	ND	ND	+	+	+	+	+	+	+	+	+	+
CPC1673-b	ND	ND	+	+	+	+	+	+	+	+	+	+
Cara	R ^w	R ^x	+	+	+	+	+	+	+	+	+	+
Alcmaria	R ^y	R ^z	–	+	+	+	+	+	+	+	+	+
Multa	R ^w	ND	–	–	+	+	+	+	+	+	+	+
SH83	R ^w	R ^x	–	–	–	+	+	+	+	+	+	+
Amaryl	R ^y	R ^z	–	–	–	+	+	+	+	+	+	+
Marijke	R ^y	R ^z	–	–	–	+	+	+	+	+	+	+
Saturna	S ^w	S ^z	–	–	–	–	–	–	–	–	–	+
RH89	S ^w	S ^x	–	–	–	–	–	–	–	–	–	–

^v Presence or absence of a CAPS (cleaved amplified polymorphic sequence) marker band that cosegregates with resistance in populations S1-Cara and FISH×RH is indicated by “+” or “–”, respectively. The order of the presented CAPS markers corresponds to the marker order on chromosome 12. ND = not determined.

^w As determined by cyst counts on at least three replicates.

^x As determined by a slot blot assay on at least three replicates.

^y Data from Arntzen et al. (1994).

^z Data from Anonymous (1991), “R” indicates extreme resistance.

neither for *Gpa2* nor for *Rx*. Clone *S. tuberosum* subsp. *andigena* CPC 1673 was collected in the late 1940s in the Andes region in South America during a search for nematode resistance (Ellenby 1952). Only five out of more than 1,200 lines belonging to more than 60 species harbored nematode resistance. The resistance in CPC 1673 appeared to be conferred by a single gene, *H* (later called the *H1* gene), that was discovered by screening populations derived from selfings of the original CPC 1673 clone (Toxopeus and Huijsman 1953; Huijsman 1955). *H1* confers resistance to *G. rostochiensis*, for which a gene-for-gene relationship has been established (Janssen et al. 1991), and the gene has been mapped on chromosome 5 (Gebhardt et al. 1993; Pineda et al. 1993). The occurrence of a second nematode *R* gene at an unlinked position on the genome of one and the same wild species clone may indicate that both *H1* and *Gpa2* may have a common ancestor, like the *L6* and *M* genes in flax (Anderson et al. 1997).

Studies on *Rx* resistance have shown that it can be considered mechanistically different from HR types of plant resistance responses. The *Rx*-mediated response has been described as "extreme resistance." It is not associated with a localized necrotic response at the site of infection (Adams et al. 1986; Köhm et al. 1993) but mediates inhibition of virus replication after having been triggered by the coat protein of PVX (elicitor of *Rx* resistance; Bendahmane et al. 1995). Nevertheless, *Rx* could still be involved in a mechanism that suppresses the viability of plant cells as plants with an *Rx* background could not be transformed with the coat protein gene of an *Rx*-avirulent PVX strain (Gilbert et al. 1998). A common feature in virus and nematode pathogenesis is that both types of pathogen are able to interfere with the plant cell metabolism. Cyst nematode juveniles penetrate the roots and migrate to the vascular cylinder, where they initiate the redifferentiation of a procambial cell into a feeding site (Williamson and Hussey 1996). Although the mechanism of *Gpa2*-mediated resistance has not yet been thoroughly investigated, the severe reduction in cyst number indicates that the nutrient flow through the nematode feeding site may be reduced in *Gpa2* plants. Poor feeding site development can be due to a localized necrosis near or at the syncytium, as demonstrated for the *H1* gene (Rice et al. 1985). In view of their tight physical linkage, *Gpa2* and *Rx* may share common components within their signal-transduction pathways, as is the case for *Pto* and *Fen*. The *Pto*- and *Fen*-mediated responses both depend on *Prf*, a third and unrelated gene that occurs in the *Pto/Fen* gene cluster (Salmeron et al. 1996). Whether the poor feeding-site development in the *Gpa2*-resistance response is related to either an *Rx*-like response or to localized necrosis near or at the syncytium, as in the case of, e.g., the *H1* gene (Rice et al. 1985), remains to be investigated.

MATERIALS AND METHODS

Plant material.

The tetraploid ($2n = 4x = 48$) progeny of selfed Cara (S1-Cara) was initially constructed for fine mapping of *Rx* (Bendahmane et al. 1997). This population was extended to a total of 2,788 genotypes. In addition, a diploid mapping population ($2n = 2x = 24$) derived from a cross between the diploid clones SH83-92-488 and RH89-039-16 was investigated. This population, encoded F1SH×RH, has been used for

mapping the *Gpa2* gene (Roupe van der Voort et al. 1997) and consisted of a total of 598 genotypes. The resistant parent SH83-92-488 and the susceptible parent RH89-039-16 will be referred to as SH83 and RH89, respectively.

Seed from a selfed offspring of *Solanum tuberosum* subsp. *andigena* CPC 1673 encoded CPC 3520 was obtained from the Commonwealth Potato Collection, Scottish Crop Research Institute, Dundee, UK. The cultivars Multa, Alcmaria, Amaryl, Marijke, and Saturna, having *S. tuberosum* subsp. *andigena* CPC 1673 in their pedigree, were derived from the collection of the Centre for Plant Breeding and Reproduction Research (CPRO-DLO) Wageningen, The Netherlands. It is noted that the background of parent SH83 contains cultivar Amaryl.

Resistance tests.

The PVX resistance test was carried out with a cDNA of the PVX_{CP4} isolate (Goulden et al. 1993). Potato plants were graft-inoculated with scions of *Lycopersicon esculentum* cvs. Ailsa Craig or Money Maker systemically infected with PVX_{CP4}. Dot blots were prepared from total RNA isolated from newly formed potato shoots 3 to 4 weeks post inoculation (Bendahmane et al. 1997). Extreme PVX resistance or susceptibility was determined by the presence or absence of a hybridization signal on dot blots probed with ³²P-labeled cDNA of PVX_{CP4} (Chapman et al. 1992). Three replicates per genotype were assayed.

For the *Gpa2* test *G. pallida* population Pa₂-D383 was used (Roupe van der Voort et al. 1997). The nematode resistance assays were performed on plants derived from in vitro stocks, stem cuttings, or tubers. In vitro plants were transferred from MS (Murashige-Skoog) medium containing 3% saccharose to a mixture of silversand and sandy loam under a moist chamber for 1 week. Two to 4 weeks after planting, plants showing vigorous growth were inoculated with nematodes. Assays were further performed as described for stem cuttings and tubers (Roupe van der Voort et al. 1997). Population Pa₃-Rook with virulence characteristics different from those of Pa₂-D383 (Bakker et al. 1992) was used to confirm the specificity of *Gpa2* resistance in tested plants. Virus and nematode resistance tests were applied on the parental genotypes as well as the S1-Cara and F1SH×RH genotypes that showed a recombination event in the GP34-IPM5 marker interval.

Construction of the potato BAC libraries and BAC library screening.

The preparation of the Cara BAC library consisting of 160,000 clones is described in detail in Kanyuka et al. (*in press*). The SH83 BAC library was prepared essentially as described in that paper with the following modifications. High-molecular-weight potato DNA was prepared in agarose plugs from potato nuclei as described in Liu et al. (1994). Half of each plug (approximately 10 µg of DNA) was digested with 10 U of *Hind*III restriction enzyme for 1 h. Size selection was carried out in two steps. Partially digested *S. tuberosum* DNA was initially subjected to CHEF (clamped homogeneous electric field) electrophoresis at 4°C in 0.5× TBE (Tris-borate-EDTA) with a linear increasing pulse time of 60 to 90 s and a field strength of 6 V/cm for 18 h. After electrophoresis, lanes containing concatemers of bacteriophage lambda as a standard for molecular weight (Bio-Rad, Richmond, CA) were re-

Table 3. Primer sequences and thermal cycling conditions for CAPS markers in the *Rx/Gpa2* interval^z

Marker	Primers	Polymerase chain reaction conditions	Restriction enzyme
191L	5'-ACAAATTGTATAATTATAGTGATACG 5'-CAAGACATTAATTAACCAACAATGG	94°C 15 s 50°C 15 s 35 cycles 72°C 2 min	<i>EcoRI</i>
77L	5'-GCTTCTAAACTCTAAATTCAGATTC 5'-CATGTGCGGACTCGTTCTTTGTAG	94°C 15 s 56°C 15 s 35 cycles 72°C 1 min	<i>AluI</i>
77R	5'-CTCGAGGGATTGAATCCAAATTAT 5'-GGAAGCAGAATACTCCTGACTACT	94°C 15 s 57°C 15 s 35 cycles 72°C 1 min	<i>HaeIII</i>
111R	5'-CCACTGTGTAAGGGTCAACTATAGTC 5'-GAGATGAAGATTTCTTGCTGTAGTG	94°C 15 s 56°C 15 s 35 cycles 72°C 1 min 30 s	Allele specific
73L	5'-CATTTCTCTGAATTGCTTCCGACTTC 5'-CCATGAAAATTGTTATCACTGAGGTG	94°C 15 s 60°C 15 s 35 cycles 72°C 1 min	<i>AluI</i>
218R	5'-GATTACAGTTGTGAATTAGTTCGGTA 5'-GCAACAGATATATCCACTTACCATTG	94°C 15 s 56°C 15 s 35 cycles 72°C 1 min 30 s	<i>AluI</i>

^z Information on additional CAPS (cleaved amplified polymorphic sequence) markers in the *Rx/Gpa2* interval is given in Bendahmane et al. (1997).

moved and stained with ethidium bromide to locate the region of the gel containing potato DNA fragments ranging from 100 to 150 kb in size. This region was excised from the gel with a glass coverslip and subjected to a second size selection step in a 1% SeaPlaque (low-melting point) agarose gel (FMC, Rockland, ME). CHEF electrophoresis was carried out for 10 h at 4°C with a field strength of 4 V/cm and a constant pulse time of 5 s. The compression zone containing DNA fragments of 100 kb was excised from the gel and dialyzed against 50 ml of TE (Tris-EDTA) for 2 h at 4°C. Dialyzed agarose slices were then melted at 70°C for 5 min and digested with 1 unit of GELASE (Epicentre Technologies, Madison, WI) per 100 mg of agarose gel for 1 h at 45°C. Ligation of the size-selected DNA to *HindIII*-digested and dephosphorylated pBeloBAC11 (kindly provided by H. Shizuya, University of Southern California, Los Angeles; Shizuya et al. 1992) and subsequent transformation of ElectroMAX *Escherichia coli* DH10B competent cells (Life Technologies, Breda, The Netherlands) with the ligated DNA was carried as described in Kanyuka et al. (*in press*), with the Bio-Rad Gene Pulser for electroporation. Settings on the Bio-Rad Gene Pulser were as recommended for *E. coli* by the manufacturer. Approximately 60,000 white colonies were picked individually into 384-well microtiter plates containing LB freezing buffer (36 mM K₂HPO₄, 13.2 mM KH₂PO₄, 1.7 mM citrate, 0.4 mM MgSO₄, 6.8 mM (NH₄)₂SO₄, 4.4% vol/vol glycerol, 12.5 µg of chloramphenicol per ml in Luria-Bertani [LB] medium), grown at 37°C for 24 h and stored at -80°C. PCR screening of the BAC libraries was carried out as described in Kanyuka et al. (*in press*).

Plant DNA marker screening.

Genomic DNA was isolated from frozen leaves or from seedlings (Bendahmane et al. 1997). CAPS markers were derived from (i) a sequenced insert of RFLP clone GP34 (kindly provided by C. Gebhardt, Max Planck Institute, Cologne, Germany), (ii) cloned AFLP markers PM3, PM4, and PM5 (coded by a "IPM" prefix; Bendahmane et al. 1997), and (iii) from the flanking sequences of BAC clones (coded by a number followed by the letter "R" or "L," for left or right terminus, respectively) isolated from the Cara BAC library (Kanyuka et al., *in press*). The end sequences of BAC insert

DNAs were isolated by inverse PCR (Ochman et al. 1990) on BAC DNA following the procedures described by Woo et al. (1994) and Kanyuka et al. (*in press*). Sequences of the clones were determined (Sanger et al. 1977) and used to design PCR primers. The DNA sequences of the PCR primers, the corresponding thermal cycling conditions, and the appropriate restriction endonuclease to identify a segregating allele in potato genomic DNA are presented in Table 3. Information on CAPS markers that are used in the present study but not mentioned in Table 3 can be found in Bendahmane et al. (1997).

Analysis of recombination.

Estimates of recombination frequencies and their standard errors were calculated with the aid of the program LINKAGE-1 (Suiter et al. 1983) by choosing the appropriate genetic model for each cross. Data for the nonrecombinant class of genotypes were set for either a 3:1 segregation ratio for the tetraploid population S1-Cara (i.e., simplex-simplex marker configuration) or a 1:1 segregation ratio for population F1SH×RH. Only strongly skewed segregation ratios will notably influence estimates of recombination frequencies (Säll and Nilsson 1994; Manly 1994). A chi-square test was used to test for pairwise differences in recombination frequencies between the marker intervals. The chi-square test criterion was determined from the recombinant and nonrecombinant classes for each marker interval. Differences (rejection of the null hypothesis) were significant when the test criterion was greater than the $X^2_{[.05]}$ value.

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