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A new gene, Ny_{tbr}, for hypersensitivity to *Potato virus Y* from *Solanum tuberosum* Maps to Chromosome IV

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Abstract A diploid backcross population derived from a cross between *Solanum tuberosum* and *Solanum berthaultii* segregated for monogenic dominant hypersensitivity to *Potato virus Y* (PVY). We propose the symbol Ny_{tbr} for this locus because plants carrying this gene develop necrosis after inoculation with PVY and the allele originated in *S. tuberosum.* The gene mapped to chromosome IV between TG316 and TG208 at LOD=2.72. This location does not correspond to any other mapped resistance genes in potato.

Keywords *Potato virus Y* · Hypersensitivity · *Solanum tuberosum* · Potato · *Potyvirus*

Introduction

Among plant families, the Solanaceae has been the focus of the most intensive effort to place loci controlling disease resistance on genetic maps. Specifically, these efforts have focused on the genera containing major crop species, *Solanum* (potato), *Lycopersicon* (tomato) and *Capsicum* (garden pepper), creating the opportunity for a comprehensive study of the organization of disease resistance genes in related crop genomes (e.g., Grube et al. 2000). Towards this end, we have worked to map a number of monogenically inherited diesease resistances in solanaceous species with particular focus on resistance to the largest family of plant viruses, the Potyviridae. The type member of this genus, *Potato virus Y* (PVY), is the most agriculturally important virus infecting the potato crop, causing heavy yield losses up to 80% (Hooker 1977; Beemster 1987). Resistance genes that control PVY have been identified in cultivated and wild potato species and have been used in potato breeding for many years (e.g., Cockerham 1955; Jones 1990). The "extreme resistance" gene(s) (Ry_{adg}, and Ry_{sto}), i.e., resistance genes that confer complete resistance to infection at the whole plant level and that are not associated with the presence of a necrotic response to the virus (Valkonen 1997), have been mapped on chromosome XI by Brigneti et al. (1997) and Hamalainen et al. (1997), respectively. At present, there is no evidence as to whether Ry_{adg}, and Ry_{sto} represent different sources of allelic genes or distinct, linked loci.

In contrast to viral resistance genes at potato loci designated *R* for extreme resistance, potato plants that carry the gene(s) for a hypersensitive response to the virus develop some necrosis after inoculation. This necrosis may be limited to inoculated tissue or may spread through the plant resulting in plant death (De Bokx and Huttinga 1981; Jones 1990; Valkonen 1994; Bawden 1936). A limited necrotic response may lead to resistance to the virus, while a systemic response will cause total crop loss. In either case, this response will limit the spread of the virus to other plantings. Loci that control this type of reaction are designated *N* genes according to the conventions for potato (Valkonen et al. 1996). No gene for hypersensitivity to PVY in potato has been reported or mapped to date although this response to PVY was noted as early as 1936 (Bawden 1936). The objective of this study was to screen parents of an existing mapping population with PVY to determine whether a differential response could be observed and, if so, to map the genes responsible for the differential phenotype.

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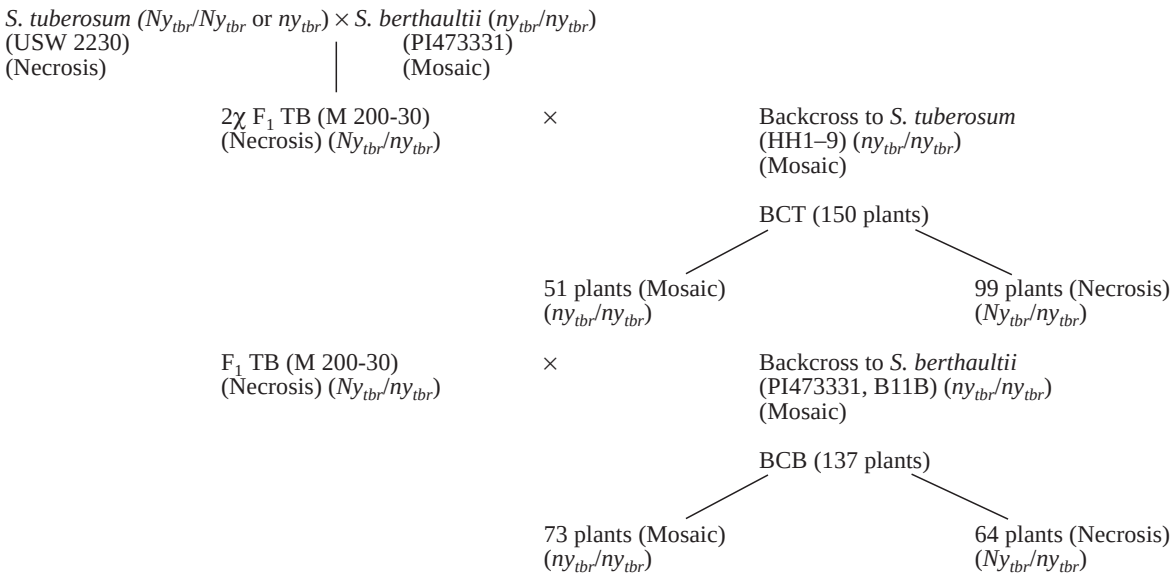

Fig. 1 The diploid mapping populations used in this study including the backcross to *Solanum tuberosum* (BCT) and the backcross population to *S. berthaultii* (BCB) were made by Bonierbale et al. (1994). This diagram illustrates population structure for the inheritance and mapping studies of hypersensitivity to PVY and proposed genotypes at the Ny_{tbr} locus. Potato clones involved in the pedigrees are: USW2230=diploid (2*x*=2n=24) derived from tetraploid cv Saco (4*x*=2n=48); PI473331=USDA accession of *S. berthaultii*; HH1–9=selected for male fertility from an intermated population of *S. tuberosum* haploids (Sanford and Hanneman 1982); B11B=clone of USDA accession PI473331. Response of clones to inoculation with PVY is shown in parentheses

Materials and methods

Germplasm and populations for genetic studies

An interspecific F_1 population was generated from a cross between two diploid potato clones, *Solanum tuberosum* USW2230 (2*x*=2n=24) derived from the tetraploid variety 'Saco' (4*x*=2n=48) and *Solanum berthaultii* PI473331. The F_1 from this cross was used as the female and backcrossed to each parental species, as described previously (Bonierbale et al. 1994) and illustrated in Fig. 1. For the male parent of the *S. tuberosum* backcross (BCT), *S. tuberosum* clone HH1–9 was used. This clone was developed from an intermated population of *S. tuberosum* haploids that had been subjected to selection for male fertility (Sanford and Hanneman 1982). For the *S. berthaultii* backcross (BCB), *S. berthaultii* clone B11B was used as the male parent. BCF_1 populations of 150 and 137 plants for BCT and BCB, respectively, were derived from reciprocal backcrosses between *S. tuberosum* and *S. berthaultii* (Fig. 1). This population has been maintained clonally in vitro as plantlets by Dr. Elmer Ewing (Cornell University, Ithaca, N.Y.). For this study, the plantlets were planted in flats, placed in a mist chamber for a week, then transplanted into 10-cm pots. Parent clones and clones from each individual in the backcross populations were transferred to a greenhouse for disease screening. Plants were maintained in the greenhouse at approximately 16° to 22°C with a 16-h photoperiod.

Viral isolates and inoculation

The PVY isolate used in this study is included in the strain group (PVY°) and was originally isolated from *S. tuberosum* clone MexSS 1035 (PI 383471) and maintained by Dr. S. A. Slack in *S. tuberosum* PI383471. Relative virus concentration in plantlets was monitored by ELISA to assure the high viral titer in tissue used for inocula. For mechanical transmission of PVY, young potato plants (in vitro plantlets less than 4 weeks after transplanting to the greenhouse) with approximately six leaves were shaded for a total of 3 days, from 1 day before through 1 day after inoculation. Six to nine plants of each genotype were inoculated with PVY° per experiment and experiments were conducted twice. Inocula were prepared as follows: sap from PVY infected potato leaves was extracted with a mechanical grinder and the extract was diluted 1:10 with phosphate buffer (1.47 mM KH_2PO_4, 8.1 mM Na_2HPO_4-anhydrous in 1 l of dH_2O, pH 7.4). Inoculations were performed as follows: carborundum (250 to 400 mesh) was dusted lightly onto plant leaves, and inoculum was applied with a cotton swab on the upper side of two leaves per plant. Inoculated leaves were marked by piercing two holes in them. Two plants of each genotype and two tobacco plants were inoculated with phosphate buffer as negative controls. Two tobacco plants and susceptible potato cultivars were used as positive controls for inoculation.

ELISA

Three samples consisting of (1) inoculated leaves, (2) two leaves two nodes above the inoculated leaves representing new growth, and (3) apical leaves were taken per plant, 2 and 4 weeks after inoculation. Samples were scored visually for symptoms and then tested by a double antibody sandwich enzyme-linked immunosorbent assay (DAS-ELISA) (Clark and Adams 1977). Monoclonal alkaline phosphotase-conjugated antibody to PVY° was obtained from Agdia (Elkhart, Ind.).

RFLP analysis and map construction

Potato leaf tissue samples from parental clones and a total of 287 individuals from both backcross progenies, BCT and BCB, were harvested for DNA extraction as described by Bernatzky and Tanksley (1986), except that sodium bisulfite (3.8 g/l) was used instead of mercaptoethanol. Parental genomic DNAs were digested with restriction enzymes *Eco*RI, *Eco*RV, *Hin*dIII, *Dra*I, *Xba*I, *Bam*HI, *Hae*III, *Taq*I, *Bst*NI, *Bgl*II, and *Sac*I (Promega and Bio-Lab), and digested DNA was loaded and separated on a 1% agarose gel in 1×TAE buffer (0.04 M Tris-acetate and 0.001 M EDTA) using electrophoresis. Gels were then subjected to Southern analysis as described by Sambrook et al. (1989). Probes TG123, TG208 and TG244 were hybridized to these filters, and results were com-

Table 1 Segregation data for hypersensitivity to PVY in a diploid potato population derived from *S. tuberosum* and *S. berthaultii*

Populations	No. of plants		Expected ratio	χ^2	Goodness-of-fit *P* values
	Necrosis	Mosaic			
S. tuberosum (USW2230)	9	0			
S. berthaultii (PI473331)	0	9			
(T×B) F_1M200–30	9	0			
S. tuberosum (HH1–9)	0	9			
S. berthaultii (B11-B)	0	9			
F_1×*S. tuberosum* (HH1–9) (BCT)[a]	99	51	1:1	15.36**	<0.0001
F_1×*S. berthaultii* (B11-B) (BCB)[b]	64	73	1:1	0.591	0.44

[a] BCT=backcross to *S. tuberosum*
[b] BCB=backcross to *S. berthaultii*

pared with previous results obtained by Bonierbale et al. (1994) to ensure that mapping individuals were correctly identified in the current study. Six plants from the BCT population and five plants from the BCB population did not appear to be correctly identified and were omitted from this study.

Preliminary analyses indicated linkage with markers on chromosome IV; therefore, additional markers from the homeologous chromosomal segment in tomato were hybridized with the mapping filters to increase the marker density in the vicinity of the phenotypically defined locus. RFLP markers based on TG clones (tomato random genomic DNA) and CT clones (tomato leaf epidermal cDNA) were provided by Dr. S.D. Tanksley, Cornell University, Ithaca, N.Y.

All filters were pre-hybridized overnight in 50–70 ml of hybridization buffer at 65°C. The probes were amplified by the polymerase chain reaction (PCR) and then radiolabelled following the manufacturer's instruction (Stratagene). Hybridization was carried out overnight at 65°C on a 50 rpm shaker. Filters were washed twice using low stringency (2×SSC and 0.1% SDS) and twice using moderate stringency (1×SSC and 0.1% SDS) at 65°C and placed on Kodak XAR-5 film and stored at –80°C for 1–15 days depending on signal strength. Films were developed using a Kodak X OMAT automatic developer system.

Data analysis

Genotypic and phenotypic segregation data collected in this study were integrated into the data set created by Bonierbale et al. (1994) for all individuals whose identities had been verified. χ^2 goodness-of-fit tests were used for preliminary analysis of segregation to detect skewing. Linkage analysis was performed using the MAPMAKER software, and loci were placed with a minimum LOD score of 3.0 and a maximum recombination fraction of 0.3 (Lander et al. 1987). The most likely order for linkage groups was tested with the "ripple" command. The Kosambi mapping function was used to calculate genetic distance in centiMorgans (Kosambi 1944). The QGENE program (Nelson 1997) was used to compare phenotypic data with genotypic data. Genotypic markers in the progeny were established by Bonierbale et al. (1994) and compared with phenotypic data for PVY.

Results

Viral screens of parental clones

The parents of the BCT population and all 150 segregants sustained PVY replication in inoculated leaves. Of these, the backcross male parent, S. *berthaultii* P1473331 clone B11B, the *S. tuberosum* backcross parent, HH1–9, and 51 progeny plants went on to become systemically infected and expressed mosaic symptoms (Fig. 1). The original S. *tuberosum* parent (USW2230), progeny from the interspecific F_1 clone M200–30, and 99 plants also became systemically infected. In contrast, these plants expressed both local necrosis and top necrosis. Most plants that developed this necrotic response died (Table 1, Fig. 2).

In the BCB population, a total of 137 plants were inoculated with PVY° and all progeny sustained PVY replication in inoculated leaves. Of these, 73 plants became systemically infected and expressed mosaic symptoms. ELISA values from these plants were clearly positive (Fig. 2). In contrast, 64 plants developed necrotic local lesions on inoculated leaves. Top necrosis was frequently observed in these plants and low levels of viral antigen were present (Fig. 2). By 4 weeks post-inoculation, most of these 64 plants had died (Table 1, Fig. 2).

When segregation data from the two backcross populations were analyzed, results from the BCB population were consistent with a 1:1 ratio (*P*=0.44) expected under the hypothesis that the gene controlling the necrotic reaction to PVY showed monogenic dominant inheritance and originated in *S. tuberosum* (USW2230). In contrast, segregation observed in the BCT population was highly skewed toward the systemic necrotic phenotype. Extreme skewing towards the *S. tuberosum* parental genotype was also previously observed in this region of chromosome IV (Bonierbale et al. 1994).

Taken together, our data from parental, F_1 and backcross populations were most consistent with the hypothesis that a single dominant gene from *S. tuberosum* (USW2230) controls hypersensitivity to PVY. *S. tuberosum* (USW2230) and the F_1 population derived from a single F_1 clone uniformly exhibited this necrotic response to PVY, while *S. tuberosum* backcross parent HH1–9 and the *S. berthaultii* parents did not.

We propose the symbol Ny_{tbr} for this locus in accordance with the conventions for potato viral resistance genes where *N* indicates a necrotic response to the pathogen was observed, *y* indicates viral pathogen PVY, and the subscript refers to the species in which the allele originated, *S. tuberosum*.

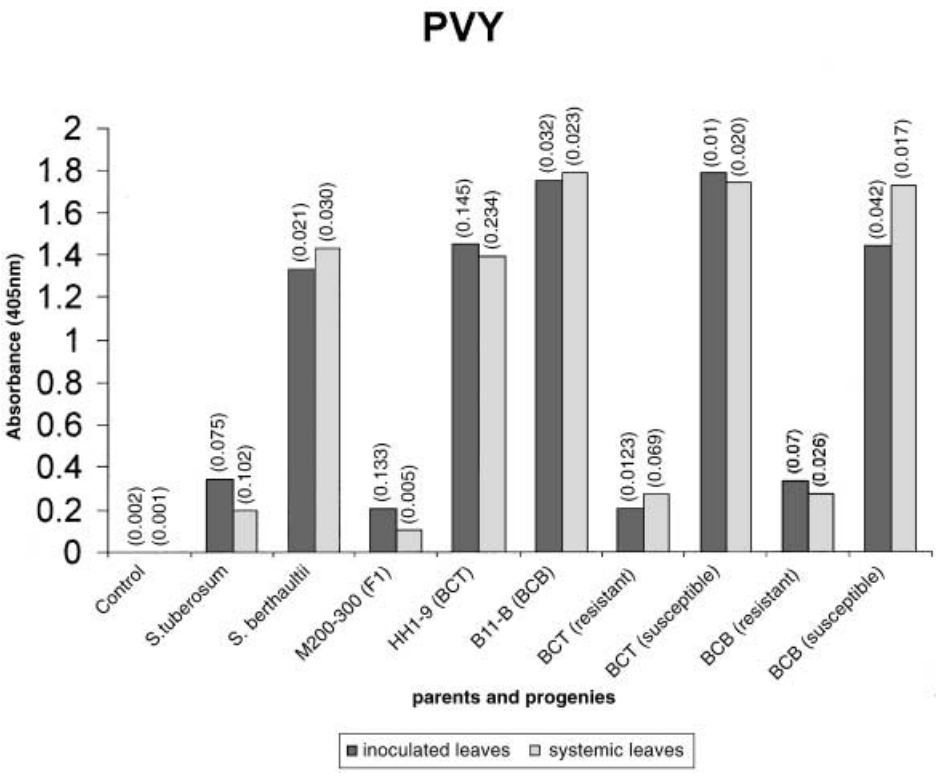

Fig. 2 ELISA values for diploid *Solanum* parental genotypes and derived materials inoculated with PVY. ELISA values were averaged from 2–4 weeks post-inoculation. BCT refers to the backcross to S. *tuberosum*, BCB refers to backcross to the S. *berthaultii*. From each parent and progeny 6–9 individual plants were mechanically inoculated. The first bar indicated for each genotype inoculated leaves and the second bar indicates values for systemically infected leaves. Mock-inoculated control values were too small to be visible in this chart (0.001 and 0.0005). Numbers in parentheses at the top of each bar indicate the standard deviation

Identification of markers linked to Ny_{tbr}

Genotypic data had been previously obtained for BCB and BCT populations by Bonierbale et al. (1994). Our genotypic and phenotypic data were added to the existing data file for this population and analyzed using QGENE (Nelson 1997) to find associations between the phenotypic data and genotypic data. Associations between the dominant locus defined by the hypersensitive response from *S. tuberosum* (USW2230) were observed with TG123 and TG208, both located on chromosome IV. Because the potato and tomato genomes are very closely related (Tanksley et al. 1992), additional RFLP markers from tomato chromosome 4 were mapped. TG623, TG75, TG146, TG182, TG268, TG287, TG339, TG432, TG474, TG483, TG519, TG 609, TG633, TG652, CT261, CT 157, CT192, CT194, CT259, CD70 and CT269 yielded no polymorphism in our mapping population. The following markers were polymorphic with the enzyme indicated: CT50 (*Eco*RI), TG370 (*Hin*dIII), TG49 (*Hin*dIII), CT175 (*Hin*dIII), TG506 (*Eco*RV), TG316 (*Dra*I), TG123 (*Hin*dIII), TG208 (*Hin*dIII) (Fig. 3). In addition to tomato and potato markers, we also wanted to determine whether the Ny_{tbr} gene was linked to the previously characterized *Ry* gene(s) in potato (Brigneti et al. 1997; Hamalainen et al. 1997) by using specific RFLP markers from chromosome XI. No linkages were observed between Ny_{tbr} and molecular markers from chromosome XI linked to the *Ry* locus or loci.

The Ny_{tbr} locus was placed on chromosome IV between TG316 and TG208 at LOD=2.72 when the data were analyzed using the Mapmaker program (Fig. 3). The closest marker linked to Ny_{tbr} was TG506 (maximum genetic distance estimated to be 3.4 cM). On the other side of Ny_{tbr}, the closest marker is TG208 with a map distance of 12.4 cM.

Discussion

A newly identified gene from S. *tuberosum* (USW2230) for dominant hypersensitivity to PVY° mapped to chromosome IV in a backcross population between diploid *Solanum* clones (Fig. 3). This locus is clearly distinct from previously reported loci in potato that control the outcome of PVY infection based on both phenotype and map position. In one of the two backcross populations analyzed in this study that is segregating at this locus, the BCB population, segregation was consistent with the hypothesis that hypersensitivity to PVY controlled by Ny_{tbr} is monogenic dominant. In the other backcross population analyzed, BCT, deviation from the expected ratio was observed, in that the susceptible class (mosaic) was smaller than expected. Because segregation distortion has previously been shown in the BCT population in this segment of chromosome IV (Bonierbale et al. 1994), we consider this the most-likely explanation for the overrepresentation of the *S. tuberosum* allele in this population. An alternative explanation for the distorted segregation observed in the BCT progeny could be that allele combinations at some loci might reduce the viability of fitness of the gamete or zygotes carrying the expected phenotype (Gebhardt et al. 1991). This has been shown

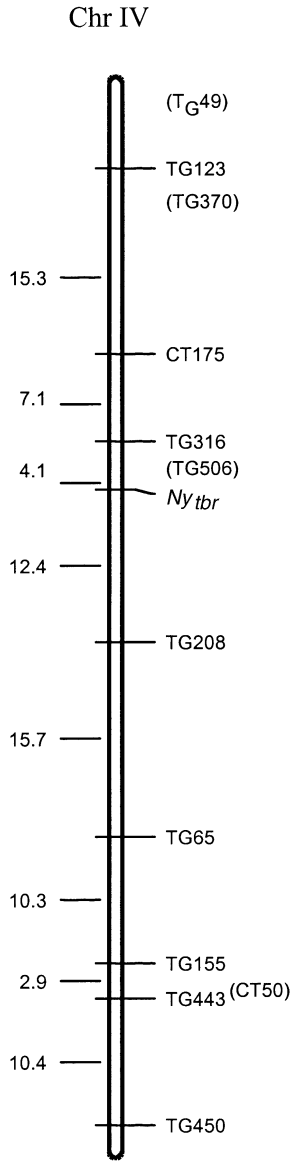

Fig. 3 Genetic map of chromosome IV showing position of the Ny_{tbr}. The RFLP markers in parenthesis are placed in intervals at LOD<3

to occur in many interspecific populations in potato (Valkonen et al. 1994; Watanabe et al. 1994) resulting in segregation distortion of resistance genes (Ritter et al. 1991; Leister et al. 1996).

Historically, the demarcation between viral resistance genes in potato that are necrotic and those that are not has been regarded as an immutable property of the gene itself; hence, the convention for designating loci (Valkonen et al. 1996). Recently, however, Bendahmane et al. (1999) showed that the nature of the resistant response, extreme resistance or hypersensitivity, could be affected by expression of the coat protein (CP) elicitor. According to their model, the appearance of necrosis during the viral-host interaction is a consequence of timing, other interacting components, and affinity between the elicitor and its receptor. Extreme resistance occurs when there is high affinity between the elicitor and its receptor, and/or when elicitor production occurs early in the infection cycle. The local hypersensitive response takes place when the elicitor has a lower affinity for the receptor, and/or when elicitor production occurs later in the infection cycle. A systemic hypersensitive response is essentially similar and occurs when there is an extremely low affinity interaction between the elicitor and the receptor, and/or when elicitor production occurs at a late stage in the infection cycle, which would account for the phenotype we observe when the Ny_{tbr} allele is present.

In fact a necrotic reaction to PVY in potato was first reported almost 70 years ago when Bawden showed that PVY caused top necrosis and eventual plant death (Bawden 1936). This condition was typically less severe under field conditions, apparently because environmental effects may mitigate this phenotype. Under the conditions of this study where large amounts of inoculum are applied, Ny_{tbr} shifts the host response from mosaic to systemic necrosis/death. Under field conditions, however, this gene may confer a useful resistance response and, in any case, use of this gene in agriculture would preclude spread of the virus; thus, this type of resistance may be useful in potato breeding since the infection is arrested and, in the field, the disease impact may be minimized.

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