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Mapping of the gene Nx_{phu} that controls hypersensitive resistance to potato virus X in *Solanum phureja* IvP35

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Abstract The line IvP35 of the diploid (2n = 2x = 24) cultivated potato species *Solanum phureja* (family Solanaceae) expresses hypersensitive resistance (H) to potato X potexvirus (PVX). In this study, a diploid potato population was produced using IvP35 as the male parent and a diploid line of *S. tuberosum* (87HW13.7) as the female parent and tested for resistance to PVX. Data indicated that H to PVX in IvP35 is a dominant, monogenically inherited trait controlled by a single gene, named Nx_{phu}, that is in a simplex condition (*Nxnx*). RFLP analysis carried out on the progeny lines revealed 4 markers (CT220, TG328, CT112 and TG424) from the long arm of chromosome IX that were linked to the hypersensitive phenotype; the closest linkage was observed with the marker TG424. Previous authors have shown that the same region of chromosome IX contains the gene *Sw-5* for resistance to tomato spotted wilt tospovirus in *Lycopersicon peruvianum* (Solanaceae).

Key words Potato virus X · Resistance gene · Genetic mapping · RFLP · Solanaceae

Introduction

Potato X potexvirus (PVX) is one of the most widespread and harmful viruses infecting potato (Hooker 1981). It is transmitted to new crops mainly via seed tubers, and therefore can be controlled using virus-free seed potatoes and by avoiding contamination from infected plants and tools. However, the use of resistant cultivars is the most efficient method for controlling PVX. The types of monogenically inherited, dominant resistance to PVX utilized in potato breeding are extreme resistance (E) and hypersensitive resistance (H) (Ross 1986). In the potato plants expressing E to PVX, no symptoms and no detectable amounts of PVX are observed in the mechanically inoculated leaves, or in the upper leaves following graft-inoculation. In contrast, H to PVX is expressed as the development of necrotic lesions in the mechanically inoculated leaves, and necrotic symptoms develop in the upper leaves following graft-inoculation (Ross 1986; Valkonen et al. 1996).

There are several dominant genes that confer resistance to PVX in the currently grown cultivars of the 'Irish potato' (*Solanum tuberosum* L.) (Ross 1986; Valkonen et al. 1996), and the chromosomal locations of few of them have been identified. The genes *Rx1* and *Rx2* control E that is effective against all strain groups of PVX (Cockerham 1970) and have been located to the chromosomes XII and V, respectively (Ritter et al. 1991). The genes for H to PVX are strain-group specific: the gene *Nb* confers resistance against PVX strains of the strain groups 1 and 2, whereas the gene *Nx* confers resistance against PVX strains of the strain groups 1 and 3 (Cockerham 1955). Recently, *Nb* has been localized to chromosome V to the same region that contains *Rx2* (De Jong et al. 1997).

Expression of H to PVX has also been reported in many other cultivated and wild potato species (*Solanum* spp.) and is monogenically inherited, similar to the genes *Nb* and *Nx* of *S. tuberosum* (Cockerham 1970; Valkonen et al. 1996). *Solanum phureja* Juz. et Buk. is one of the several potato species cultivated in the Andean region of South America (Hawkes 1990). It is diploid (2n = 2x = 24), and a few genotypes such as

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IvP35 are also used as pollinators for haploid induction by potato breeders (Hermsen and Verdenius 1973). IvP35 expresses characteristic H to the PVX strain group 3 (Valkonen et al. 1995), which is hypothesized to be controlled by an *Nx* gene, named Nx_{phu}. The aim of the study described here was to test the expected monogenic, dominant inheritance of H to PVX in IvP35 and to identify the chromosomal region that contains the resistance gene.

Materials and methods

Plant material

A diploid (2n = 2x = 24) potato population was produced by crossing the diploid *S. tuberosum* line 87HW13.7 (female parent; Watanabe et al. 1995) with the diploid *S. phureja* line IvP35 (male parent; Hermsen and Verdenius 1973) under controlled greenhouse conditions at Cornell University, Ithaca, N.Y., USA. The parental lines were provided by the International Potato Center (CIP), Lima, Peru.

The parental lines and 109 progeny were tested for resistance to a strain group-3 isolate of PVX (PVX-UK; Valkonen et al. 1994) by graft-inoculation as previously described (Valkonen et al. 1995). Expression of resistance was detected as the development of many necrotic lesions in the upper leaves 12–17 days after graft-inoculation. Symptomless plants were tested for PVX by ELISA using polyclonal antibodies to PVX (Boehringer-Mannheim, Germany) as previously described (Valkonen et al. 1994).

Restriction fragment length polymorphism (RFLP) analysis

DNA extraction (Bernatzky and Tanksley 1986), bulked segregant analysis (BSA) (Michelmore et al. 1991) and RFLP analysis (Sambrook et al. 1989) were done as previously described (Hämäläinen et al. 1997). For BSA, two DNA pools were prepared: one contained DNA of 12 progeny expressing H to PVX, and the other one consisted of DNA of 12 susceptible (symptomlessly infected) progeny. Bulked DNA was digested with *Bst*NI, *Dra*I, *Eco*RI, *Eco*RV, *Hae*III, *Hin*dIII, *Msp*I, *Pst*I, *Taq*I or *Xba*I. DNA fragments were separated on 1.0% agarose gels and blotted onto nylon filters (MAGNA, Micron Separations).

A total of 70 potato and tomato genomic and cDNA clones were used as probes in the RFLP analysis (Gebhardt et al. 1989; Tanksley et al. 1992). They were chosen at approximately 10-cM intervals throughout the potato genome. Probes were labeled with digoxigenin and hybridized to the above-mentioned filters containing potato DNA. The signal was detected by chemiluminescence following the manufacturer's instructions (Boehringer Mannheim, Germany). RFLP analysis of 86 individual progeny tested for resistance to PVX (47 hypersensitive progeny and 39 susceptible progeny) was carried out with the putatively polymorphic markers as previously described by Hämäläinen et al. (1997). Linkage analysis was carried out using the MAPMAKER/EXP 3.0 software (Lander et al. 1987).

Results

The male parent IvP35 and 54 progeny expressed H to PVX, and many necrotic lesions developed in the top leaves. The female parent 87HW13.7 and 55 progeny showed no symptoms, but they contained high titres of PVX detected by ELISA and were susceptible to PVX. These data indicated that H to PVX is monogenically and dominantly inherited and that the gene Nx_{phu} in IvP35 is in a simplex condition (*Nxnx*).

BSA was first carried out using RFLP markers located on the potato/tomato chromosomes V, VI, XI and XII that are known to contain several disease resistance genes in potato (reviewed in Leister et al. 1996). Eight to twelve markers from each chromosome were used for screening, but no polymorphism between the H and S pools was observed. Subsequently, markers from other chromosomes were tested, and 4 markers from chromosome IX (CT220, *Eco*RV digest; TG328, *Eco*RI digest; CT112, *Taq*I digest, TG424, *Eco*RV digest) showed polymorphism in BSA. With all 4 markers, a band of 2–6.5 kbp was identified that was observed in the H pool but not in the S pool. RFLP analysis of the individual progeny and the tests for independent assortment showed that all these markers were linked to Nx_{phu} (CT220: $\chi^2 = 55.67$; TG328: $\chi^2 = 63.30$; CT112: $\chi^2 = 67.40$; TG424: $\chi^2 = 67.75$; $P = 0.005$). Among the 86 progeny tested with markers CT220, TG328 and CT112, 9, 7 and 6 recombinants were detected, respectively, but no recombinant was observed among 67 progeny tested (including the recombinants mentioned above) using TG424 as the probe. No polymorphism between the BSA pools was observed with the probe TG421 that is located in centromeric orientation from TG424 (Fig. 1). These

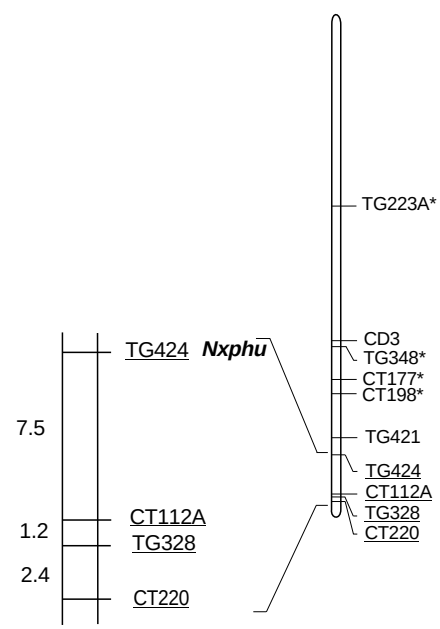

Fig. 1 Composite genetic map of chromosome IX showing the predicted position of Nx_{phu} and the markers tested in this study. Marker distances are based on segregation in an interspecific cross among diploid potatoes or, for those marked with *, among diploid tomatoes (Tanksley et al. 1992). The *underlined* markers are linked to Nx_{phu}

data indicated that Nx_{phu} is located in the long arm of chromosome IX and shows a close linkage with TG424 (Fig. 1).

Discussion

Our data show that the gene Nx_{phu} that confers hypersensitive resistance (H) to PVX in the cultivated potato species *S. phureja*, line IvP35, is located at the distal end of the long arm of chromosome IX. Its location is different from those of the other PVX resistance genes mapped so far. The gene *Nb* (H to PVX) and the gene *Rx2* [extreme resistance (E) to PVX] have been mapped to the same region of chromosome V (Ritter et al. 1991, De Jong et al. 1997), whereas the gene *Rx1* for E to PVX has been mapped to chromosome XII (Ritter et al. 1991). No other resistance genes in potato are known in the same region of chromosome IX that contains Nx_{phu}.

However, the *Sw-5* locus that confers dominant resistance to tomato spotted wilt tospovirus (TSWV) in the wild tomato species *Lycopersicon peruvianum* Mill. by retaining the plants free of systemic infection (Stevens et al. 1992) is also located on the long arm of chromosome IX (Stevens et al. 1995; Brommonschenkel and Tanksley 1997). *Sw*-5 is tightly linked to CT220 (0.15 cM; Brommonschenkel and Tanksley 1997), whereas Nx_{phu} showed the closest linkage with TG424. Thus, Nx_{phu} is located in centromeric orientation from CT220 and, consequently, the locus corresponding to *Sw-5* in *L. peruvianum*. On the other hand, the gene *Tm-2a* that confers resistance to tobacco mosaic tobamovirus (TMV) in *L. peruvianum* by restricting the cell-to-cell movement of TMV (Meshi et al. 1989) is located very close to the centromeric region of chromosome IX (Pillen et al. 1996), which also seems to be different from Nx_{phu}.

Comparative mapping on tomato and potato genomes (Bonierbale et al. 1988; Gebhardt et al. 1991; Tanksley et al. 1992; Bonierbale et al. 1994) has shown that the genomes are generally quite homeologous. No rearrangements have been found in the region of the long arm of chromosome IX that corresponds to the locations of *Nx* and *Sw-5*, which has also been shown to be true for *S. phureja* (Bonierbale et al. 1988). In contrast, differences between potato and tomato genomes are evident in the more proximal part of the long arm and in the short arm of chromosome IX (Bonierbale et al. 1988; Gebhardt et al. 1991; Tanksley et al. 1992). Although the loci of *Nx* and *Sw-5* in *S. phureja* and *L. peruvianum* may not be syntenic, it is interesting that these two virus resistance genes are located within a relatively short segment of chromosome IX in the two solanaceous species. Other chromosomal regions are also known of that are rich in resistance genes in different solanaceous species (reviewed in Leister et al. 1996). For example, the same region of chromosome XI contains a locus for resistance to *Synchytrium endobioticum* in *S. tuberosum* (Leister et al. 1996), the gene Ry_{adg} for E to potato virus Y (PVY) in *S. tuberosum* subsp. *andigena* Hawkes (Hämäläinen et al. 1997), the gene Ry_{sto} for E to PVY in *S. stoloniferum* Schlechdt. et Bche.(Brigneti et al. 1997), the root-knot nematode resistance gene R_{Mc1} in *S. bulbocastanum* Dun. (Brown et al. 1996) and the gene *N* for H to TMV in *Nicotiana sylvestris* (Whitham et al. 1994; Leister et al. 1996).

The localization of small chromosomal regions containing different resistance genes in different potato or other solanaceous species will have an economical impact on breeding programs because they may benefit from the utilization of relatively few markers for simultaneous selection of several resistance traits. Examination of the chromosomal regions that are rich in resistance genes is also interesting from the point-of-view of understanding plant and resistance gene evolution. These questions will remain a subject for our further studies.

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