# Extreme Resistance is Epistatic to Hypersensitive Resistance to Potato Virus $Y^O$ in a *Solanum tuberosum* subsp. *andigena*-Derived Potato Genotype

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**ABSTRACT**

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Resistance to potato virus Y ($PVY^O$) was examined in diploid potato progeny from a cross of two diploid, interspecific hybrids, 2X(V-2)7 (extremely resistant to $PVY^O$) and 84.194.30 (susceptible to $PVY^O$). The pedigree of 2X(V-2)7 includes *Solanum tuberosum* subsp. *andigena* (extremely resistant to $PVY^O$) and *S. t. tuberosum* (susceptible to $PVY^O$). Following inoculation, 34 $F_1$ progeny (*Ry/ry Ny/ny* or *Ry/ry ny/ny*) and 2X(V-2)7 (*Ry/ry Ny/ny*) remained symptomless and no virus was detected by ELISA, which is characteristic of extreme resistance controlled by the $Ry_{adg}$ gene. Eight progeny (*ry/ry Ny/ny*) developed leaf-drop, necrotic streaks, lesions, and mottle symptoms in systemically infected leaves, which are characteristic of hypersensitive resistance controlled by an *Ny* gene. Twenty progeny (*ry/ry ny/ny*) and 84.194.30 (*ry/ry ny/ny*) were susceptible to $PVY^O$ and developed mottle symptoms without necrosis. These results indicated that extreme resistance and hypersensitivity to $PVY^O$ were derived from *S. t. andigena* and that the expression of extreme resistance is epistatic to the expression of hypersensitivity in 2X(V-2)7.

Viruses are important pathogens of potato (*Solanum tuberosum* L.) because many of them can cause heavy yield losses and can be transmitted to new crops through vegetative seed tubers (24).

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Potato virus Y (PVY) and potato leafroll virus are the major viral pathogens of potato in most temperate areas in the world. Since PVY is transmitted efficiently in a nonpersistent manner by aphids in the field, it may cause substantial losses despite limited generation seed certification programs and other horticultural control practices. Cultivar resistance to PVY has promise as a persistent and cost-effective means of control (12,24).

According to Peloquin et al (21) and Watanabe et al (27), potato germ plasm (*S. tuberosum*; $2n = 4X = 48$) can be enhanced more efficiently by combining useful genes from various *Solanum* genotypes at the diploid level ($2n = 24$) using diploid species and haploidization of the tetraploid genotypes. Both qualitative and quantitative genetic attributes can then be transferred from diploid to tetraploid potatoes using $2n$ gametes.

Single dominant genes control hypersensitivity and extreme resistance to viruses in cultivated and wild *Solanum* spp. and have been utilized in breeding of potatoes for virus resistance (11; for review see 24 and 25). Hypersensitivity is coded for by *N* genes and is virus- or virus-strain-specific. Necrotic lesions appear on mechanically inoculated leaves, and necrosis also develops in systemically infected parts of plants. In contrast, extreme resistance controlled by *R* genes is active against a broader range of virus strains and closely related viruses. Extremely resistant plants remain symptomless following inoculation and are rarely infected. Although the *N* genes confer useful field resistance to specific viruses, the *R* genes are more desirable for their broader and more comprehensive mode of action (11,17, 23,24).

Extreme resistance to PVY, coded for by a single dominant gene, *Ry*, derived from *S. t. andigena*, has been exploited in potato breeding (13,14,19,22). Cockerham (11) suggested that *S. t. andigena* had a specific gene for hyper-

Table 1. Pedigree, species, and type of $PVY^O$ resistance in diploid ($2n = 24$) parental potato clones

| Diploid parent | Pedigree | | Resistance |
|---|---|---|---|
| | Female: Species involved | Male: Species involved | |
| 84.194.30 (CIP 590007.30) | M17.10: *Solanum tuberosum* subsp. *andigena*, *S. chacoense*, *S. phureja*, *S. sparsipilum*, *S. stenotomum*, *S. t. tuberosum* | M149.10: *S. t. andigena*, *S. chacoense*, *S. phureja*, *S. sparsipilum*, *S. stenotomum*, *S. t. tuberosum* | Susceptible |
| 2X(V-2)7 (CIP 590001.7) | V-2[a]: *S. t. andigena*, *S. t. tuberosum* | IvP35: *S. phureja* | Extremely resistant |

[a]Donor of the $PVY^O$ resistance; derived through haploidization by pseudogametic parthenogenesis.

Table 2. Segregation of extremely resistant, hypersensitive, and susceptible genotypes as determined by graft-inoculation with $PVY^O$

| Genotype | No. of plants | Phenotype | ELISA[a] | Proposed genotype[b] |
|---|---|---|---|---|
| Parents | | | | |
| 2X(V-2)7 | 6 | Extremely resistant | 0.01 ± 0.01 | *Ry/ry Ny/ny* |
| 84.194.30 | 6 | Susceptible | 1.53 ± 0.11 | *ry/ry ny/ny* |
| $F_1$ crosses | 34 | Extremely resistant | 0.00 ± 0.00 | *Ry/ry Ny/ny* or *Ry/ry ny/ny* |
| | 8 | Hypersensitive | 0.83 ± 20 | *ry/ry Ny/ny* |
| | 20 | Susceptible | 1.46 ± 0.27 | *ry/ry ny/ny* |

[a]Mean ELISA readings ± standard error 3 wk after graft-inoculation with $PVY^O$.
[b]$\chi^2$ (df = 2) = 5.23 ($0.05 < P < 0.1$).

sensitivity to PVY strain group C ($PVY^C$) and another gene for hypersensitivity to potato virus A (PVA) but did not find evidence for hypersensitivity to $PVY^O$ (9,11). In this study, we report hypersensitivity and extreme resistance to $PVY^O$, both derived from *S. t. andigena*, in a haploid potato hybrid and provide evidence that $Ry_{adg}$ is epistatic to $Ny_{adg}$ in potato genotypes where they occur together.

## MATERIALS AND METHODS

**Plant material.** The haploid ($2n = 24$) potato clone 2X(V-2)7 (CIP 590001.7) (extremely resistant to $PVY^O$) was obtained by haploid induction from tetraploid (4X) clone V-2 using IvP35 as the haploid-inducing pollinator (16) and was crossed with the diploid ($2n = 24$) potato clone 84.194.30 (CIP 590007.30) (susceptible to $PVY^O$). These parental clones were obtained from the International Potato Center (CIP), Lima, Peru, and their pedigree is shown in Table 1. The 66 hybrid seedlings obtained were multiplied by shoot cuttings and grown in 10-cm-diameter clay pots in an air-conditioned greenhouse under natural daylight during June–September. Means of the daily minimum and maximum temperatures were 18 and 28 C, respectively.

**PVY isolate.** The isolate of PVY used belonged to the ordinary strain group ($PVY^O$) and was originally obtained from *S. tuberosum* clone Mex 1035 (PI 383471) (15). It has since been maintained in tobacco (*Nicotiana tabacum* L. cv. Samsun). It is a $PVY^O$ isolate because: 1) it does not cause necrosis in tobacco (2) as distinct from the PVY strain group N isolates ($PVY^N$) that are necrotic in tobacco (4); 2) it does not cause necrosis in potato cv. King Edward carrying genes *Nc*, *Na*, and *Nv* controlling specific hypersensitivity to $PVY^C$, PVA, and potato virus V (PVV), respectively (17,26); and 3) it does not react in serological tests with monoclonal antibodies raised against $PVY^C$ and $PVY^N$ and polyclonal antibodies raised against PVA. Futhermore, it causes necrosis in potato cv. Pentland Crown carrying a specific hypersensitivity gene to $PVY^O$ (17,26). For the present study, the $PVY^O$ isolate was propagated in potato cv. Atlantic, which develops mosaic symptoms without necrosis when infected with $PVY^O$.

**Virus inoculation and detection.** Three plants of each genotype were sap-inoculated at the three- to five-leaf stage. Leaves of $PVY^O$-infected cv. Atlantic were ground with a pestle and mortar at 1g/5 ml of distilled water, and sap was rubbed onto two Carborundum-dusted leaves of each test plant. Inoculated leaves were marked with holes with the tip of a Pasteur pipette, and plants were tested by double antibody sandwich enzyme-linked immunosorbent assay (DAS-ELISA) (7) 3 wk after sap-inoculation using antisera obtained from Agdia (Elkhart, IN). The uppermost fully expanded leaves of plants were sampled and weighed, and sap was extracted at 1g/3 ml of extraction buffer in polyvinyl plastic bags. Two 100-$\mu$l aliquots were transferred from each sample to two wells of a microtiter plate coated with PVY antibodies. Absorbances were recorded at 405 nm 45 min after addition of *p*-nitrophenyl phosphate as a substrate. One plant of each genotype that was not PVY-infected as determined by DAS-ELISA was side-grafted with one scion of $PVY^O$-infected potato cv. Atlantic as described by Valkonen et al (26). Vigorous growth of the grafted scion was taken as an indication of a successful graft union. Scions were removed 16 days after grafting, and plants were tested by DAS-ELISA 21 days after inoculation as described above.

## RESULTS AND DISCUSSION

Ten of the 66 tetraploid progeny developed mild yellow mottle symptoms following sap-inoculation with $PVY^O$ and were PVY-infected as determined by ELISA. When the remaining 56 noninfected progeny were graft-inoculated with $PVY^O$, 10 developed mild yellow mottle symptoms and high PVY titers as determined by ELISA. Thus, a total of 20 progeny were susceptible to $PVY^O$ (Table 2). Eight graft-inoculated progeny developed necrotic lesions in systemically infected leaves. The width of the chlorotic halo around the necrotic center of the lesions varied greatly among the eight progeny 12 days after graft-inoculation (Fig. 1). However, 24 days after graft-inoculation, all plants had uniform systemic symptoms of leaf-drop; large, irregular-shaped necrotic lesions and vein necrosis in leaves; necrotic streaks in stems; and mottle in top leaves. Leaf-drop, necrotic lesions, and mottle symptoms continued to develop in these plants for 6 wk after the removal of $PVY^O$-infected scions. Thirty-four graft-inoculated progeny remained symptomless, and PVY was not detected by ELISA (Table 2). Four plants died during the graft-inoculation experiment. The parental clone 2X(V-2)7 remained symptomless, and no PVY was detected by ELISA after graft-inoculation, whereas 84.194.30 showed severe mottle symptoms and had a high PVY titer (Fig. 1, Table 2).

The lack of detectable $PVY^O$ and symptoms in the graft-inoculated parental clone 2X(V-2)7 and in 34 progeny is characteristic of extreme resistance controlled by *Ry* genes in *Solanum* spp. (11,24) and similar to the extreme resistance to $PVY^O$ previously described in *S. t. andigena* (13,19). On the other hand, the development of necrosis in eight graft-inoculated progeny is characteristic of the expression of hypersensitivity to $PVY^O$ coded for by *Ny* genes in potato (11,17,24). Mechanical inoculation would not have permitted the detection of *Ny* genotypes in our study. Instead, the *Ny* genotypes would have been

erroneously classified as extremely resistant because no necrotic local lesions were observed and no virus was detected after sap-inoculation. Necrotic lesions usually develop in mechanically inoculated leaves of *Ny* genotypes (17,23); but if the physiological and environmental conditions are unfavorable for development, the lesions may be few, small, and difficult to observe. In addition, the local response may prevent systemic spread of the virus (8,11). Therefore, it is important to assess the resistance response by graft-inoculation (6).

Of the 62 genotypes from which conclusive results were obtained, 34, 8, and 20 were categorized as extremely resistant, hypersensitive, and susceptible, respectively (Table 2). This response suggested that two genes for resistance to $PVY^O$ were segregating in progeny, one for hypersensitivity ($Ny_{adg}$) and the other for extreme resistance ($Ry_{adg}$). Because both resistance genes must have come from 2X(V-2)7 (as 84.194.30 was fully susceptible to $PVY^O$), the genotype designation for 2X(V-2)7 is *Ry/ry Ny/ny* and that for 84.194.30 is *ry/ry ny/ny*. The expected progeny genotype ratio from the cross of 2X(V-2)7 and 84.194.30 would be *Ry/ry Ny/ny*:*Ry/ry ny/ny*:*ry/ry Ny/ny*:*ry/ry ny/ny* = 1:1:1:1, and the phenotype ratio of extremely resistant, hypersensitive, and susceptible would be 2:1:1 (34:8:20 in our study). The observed $\chi^2$ value (df = 2) in our study was 5.23, which is consistent with the hypothesis of two resistance genes segregating in progeny ($0.05 < P < 0.1$). If the genes *Ry* and *Ny* were allelic, a 1:1:0 ratio of extremely resistant, hypersensitive, and susceptible genotypes, respectively, would have been expected in the $F_1$ progeny. The observation that 2X(V-2)7 is extremely resistant to $PVY^O$ and develops no necrosis indicates that $Ry_{adg}$ is epistatic to $Ny_{adg}$. Furthermore, about 50% of the progeny expressed extreme resistance without necrosis (equating to a 1:1 ratio for the presence and absence of the $Ry_{adg}$ gene), which suggests that the likely presence of the $Ny_{adg}$ gene in some of these genotypes did not affect the resistance phenotype. This is supported by the fact that Cockerham (11) crossed *S. hougasii* Corr. (*Ry/ry ny/ny*) with *S. demissum* Lindl. (*ry/ry Ny/ny*) and also crossed a few *Ry/ry ny/ny* and *ry/ry Ny/ny* genotypes of *S. stoloniferum* Schlechtd. & Bche. with each other, with the result that the progeny *Ry/ry Ny/ny* were indistinguishable from those of *Ry/ry ny/ny* in terms of $PVY^O$ resistance. When the *Ry/ry Ny/ny* genotypes were crossed with susceptible genotypes (*ry/ry ny/ny*), the ratios of the

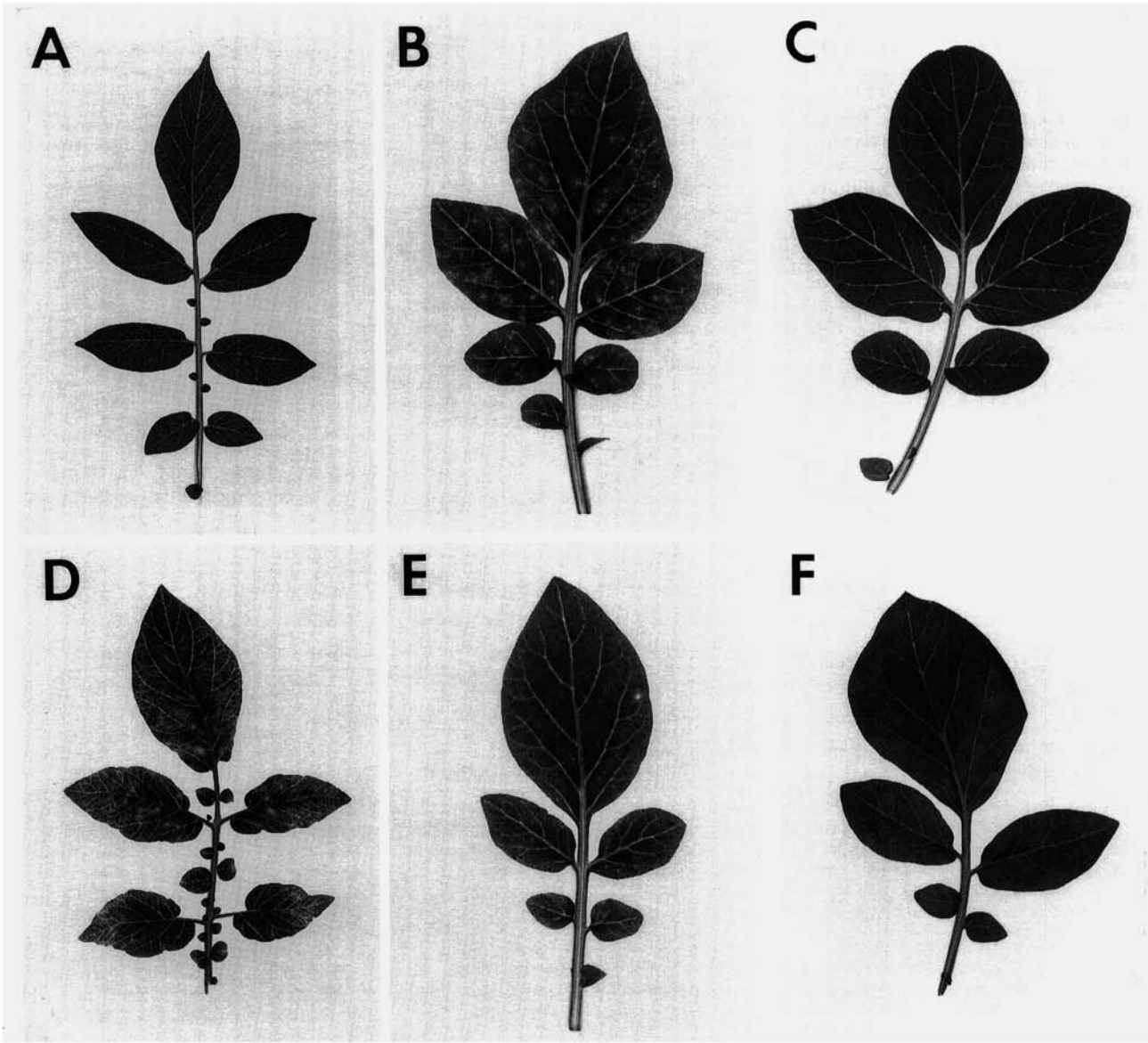


**Fig. 1.** Symptoms in systemically infected leaves 12 days after graft-inoculation with $PVY^O$: **(A)** Parental clone 2X(V-2)7 showing no symptoms and **(D)** parental clone 84.194.30 showing severe mottle symptoms; **(B)** and **(E)** progenies expressing hypersensitivity; **(C)** progeny expressing extreme resistance; and **(F)** susceptible progeny showing mottle symptoms (the two black spots were light brown naturally and were caused by mechanical damage to the leaf).

different resistance phenotypes in the progeny were similar to those we observed in the present study. We have also crossed a diploid hybrid derived from *S. stoloniferum* (*Ry*/*ry ny*/*ny*) with 84.194.30 (*ry*/*ry ny*/*ny*) and obtained a clear 1:1 ratio (33:34) of progeny extremely resistant and susceptible to $PVY^O$ (*unpublished*).

Hypersensitivity to $PVY^O$ has been observed in a few haploid genotypes obtained from *S. t. andigena* (20). The occurrence of few progeny hypersensitive to $PVY^O$ among extremely resistant and susceptible progeny in the crossing populations of *S. t. andigena* has been explained as "distorted segregation" of the extreme resistance gene $Ry_{adg}$ because the resistance was thought to function at a single locus (14; R. L. Plaisted, *unpublished*). Therefore, genetic mapping for $Ry_{adg}$ has been considered to be unattractive, because segregation of traits that would correspond to discrete phenotypes has not been expected. However, in this study we have obtained evidence for two resistance genes ($Ry_{adg}$ and $Ny_{adg}$) being donated by *S. t. andigena* instead of one resistance gene and we have observed that $Ry_{adg}$ is epistatic to $Ny_{adg}$. These findings have encouraged us to initiate molecular studies for the genetic localization and isolation of the $Ry_{adg}$ and $Ny_{adg}$ genes in order to address the more general questions of whether *N* genes in potato are mutants of *R* genes, as hypothesized by Cockerham (10,11), and/or whether these genes function principally by a common mechanism, as suggested by others (1,3,5,18).


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