Resistance to a Necrotic Strain of Potato Virus Y Among *Nicotiana* Species, Somatic Hybrids, and Tobacco Cultivars

J. E. BRANDLE, Agriculture Canada, Research Station, P.O. Box 186, Delhi, Ontario, N4B 2W9; L. W. STOBBS, Agriculture Canada, Research Station, P.O. Box 6000, Vineland Station, Ontario, L0R 2E0; and S. GLEDDIE, Agriculture Canada, Plant Research Centre, Central Experimental Farm, Ottawa, Ontario, K1A 0C6

ABSTRACT

Brandle, J. E., Stobbs, L. W., and Gleddie, S. 1995. Resistance to a necrotic strain of potato virus Y among *Nicotiana* species, somatic hybrids, and tobacco cultivars. Plant Dis. 79:152-154.

Fifty-four tobacco cultivars, seven species, and four somatic hybrids were examined for resistance to a Canadian isolate of potato virus Y necrotic strain (PVY^N). Four cultivars were resistant to infection: Virgin A Mutante (VAM), NC744, TN86, and PBD6. Virus was not detected in inoculated plants by enzyme-linked immunosorbent assay, electron microscopy, or back-inoculation to susceptible tobacco plants. Resistant plants were similar in appearance to the healthy uninoculated controls. A single recessive gene from VAM is believed to be common to the four resistant varieties. Havana 307, Wanda, and Wisana were tolerant to PVY^N infection, exhibiting mild mosaic symptoms with or without veinal necrosis, and similar in overall growth to the healthy controls. All the registered Canadian cultivars tested were susceptible to PVY^N infection. Of the species tested, *Nicotiana kawakami* exhibited mild symptoms and reduced virus titer and *N. rustica* L. var. NRT exhibited tolerance when inoculated with PVY^N. No resistance or tolerance to PVY^N infection was found in any of the somatic hybrids tested. These results demonstrate that genetic resistance to PVY^N is present both within the *tabacum* genome and among its wild relatives. The utility of the sources of resistance is variable, however.

Potato virus Y strains with the potential to cause significant crop loss in tobacco have been present in the United States for a number of years (6). Numerous strains of the virus are known, and recently, a European necrotic strain (PVY^N) was found in Canada (10,11). Infected seed potatoes were identified as one source of the virus and the imposition of export quarantines on seed potatoes led to substantial economic losses to potato growers (10). Furthermore, tobacco crop losses directly attributed to PVY^N were reported to exceed $1 million in 1989 (8).

Many members of the genus *Nicotiana* and a large number of tobacco cultivars and breeding lines of *N. tabacum* L. have been screened for resistance to the three prevalent American strains (MM, MN, and NN) of PVY (6) and to other strains found in Europe, South America, Africa, and Asia (2,6,12,15). As a result of these screening efforts, sources of resistance or tolerance have been found among *N. tabacum* cultivars and other *Nicotiana* species. The inheritance of resistance has been investigated in *N. tabacum* cultivars, but resistance found in species has not. There has not been any report of a systematic search for resistance to PVY^N. Given the destructive nature of the disease, the identification of sources of genetic resistance that can be used in PVY^N abatement is essential, and the purpose of this work was to identify potential sources that could be used in a tobacco breeding program.

Accepted for publication 20 October 1994.

MATERIALS AND METHODS

Plant material. Fifty-four tobacco cultivars, seven species, and four somatic hybrids were screened for their reaction to PVY^N. The somatic hybrids were between *N. tabacum* cultivar Delgold and *N. rustica* var. NRT, *N. debneyi* Domin., N. sylvestris Speg., and *N. glutinosa* L. (14). Seedlings of the tobacco cultivars and *Nicotiana* species were germinated for 10 days on wet filter paper in petri plates, then transferred to 5- × 5-cm peat pots filled with a 2:1 mixture of sand and muck. Somatic hybrid plants were grown from leaf explants of existing hybrids. Leaves were removed from greenhouse-grown somatic hybrids, sterilized by soaking for 1 min in ethyl alcohol (15.7 M) then 10 min in NaOCl (0.07 M), followed by three rinses in sterile distilled water. The leaves were cut into 1-cm^2 pieces, and the resultant pieces were placed abaxial surface down on MS media (5) supplemented with 0.044 M benzyl aminopurine (Sigma Chemical Co., St. Louis). Four weeks later, plantlets were removed from the margins of the leaf explants and transferred to MSB5 media (5) for rooting. Roots were established within 4 wk, and plantlets were transferred to potting medium in peat pots as described for seedlings.

Virus. The Ontario tobacco isolate of PVY^N was used in all inoculations (10). This isolate is the predominant strain occurring in tobacco and potato crops in southern Ontario. A single-lesion isolate of the virus propagated through successive transfers on *Chenopodium quinoa*, was maintained in *N. tabacum* cv. Harrow Velvet and used for all inoculations.

Virus transmission. Virus inoculum was prepared by triturating systemically infected Harrow Velvet leaves (1:9 w/v) in enzyme-linked immunosorbent assay (ELISA) extraction buffer (4) and inoculating onto Carborundum-dusted leaves of 20 plants of each of the tobacco cultivars, species, and somatic hybrids. Plants were maintained in the greenhouse (25 C) for 24 days, at which time symptoms were recorded and plants were assayed for virus.

Field studies. To observe any differences in symptom expression under field conditions, a quarantine planting was established at the Agriculture Canada Animal Disease Research Institute farm in Nepean, Ontario, in accordance with Plant Health Directorate policy. Thirty seedlings of each cultivar were sown in a sandy-silt soil on 10 June in three replicate groups planted in 30-m rows with 1-m spacing between plants in each row. Rows were 1.5 m apart. Plants were rub-inoculated with PVY^N when transplanted (10-wk-old seedlings), and standard agronomic practices were followed (1). Rows were weeded manually. Virus symptoms were recorded 20 September, and foliage was harvested from each plant for virus assays.

Virus assay. All inoculated plants were tested using direct double-antibody sandwich ELISA (DAS-ELISA), as described by Clark and Adams (4). A composite sample of uninoculated leaves was taken from each plant and triturated in ELISA extraction buffer (4) (1.9 w/v). Inoculated leaves were subsequently assayed from symptomless plants in which systemic movement of virus was not detected by ELISA. A monoclonal antibody (P. Ellis, Agriculture Canada, Vancouver, BC) prepared against the Canadian isolate of PVY^N was used in all tests. All assays were performed in Immulon 2 flat-bottomed Removawell strips (Dynstech Laboratories, Alexandria, VA) with 200 ml of reagent used for each of the four steps. Two hundred microliters of tissue triturates (tissue/ELISA extraction buffer 1:19, w/v) were loaded into three replicate wells for each sample. Alkaline phosphatase (type 7, Sigma) conjugated to PVY^N/immunoglobulin G at an enzyme/protein ratio of 2.5:1 (v/v) was added for 3 hr at 38 C. Substrate absorbance was measured at 405 nm in a Eurogenics MPR-44 microplate reader (DiaMed Laboratory Supplies, Mississauga, ON, Canada). Test samples were considered positive if their absorbance values exceeded twice that of healthy control samples.

RESULTS AND DISCUSSION

Fifty-four tobacco cultivars, seven species, and four somatic hybrids were screened for reaction to PVY^N. The following cultivars were judged to be susceptible based on symptomatology (all exhibited stunting, mosaic, mottling, and veinal necrosis in greenhouse and field trials) and positive ELISA values: Belgique, Burley 21, Candel, Coker 371 Gold, Danya #3, Delfield, Delgold, Delhi 76, Delliot, Speight G28, Speight G102, Speight G108, Speight G128, Gold Start 6007, Grande Rouge, Islangold, K 149, K 326, K 340, K 394, K 399, KY 14, KY 171, Kutsaga 110, Kutsaga E1, Little Crittendon, McNair 944, NC37NF, NC 60, NC 82, NCTG51, NK5168, Newdel, Nordel, Q269-5, RG 22, RG 8, Reams 158, S110, TB5-2, VS 16, VS 21, VS 3, VS13, Va. 115, and Va. 116. The ELISA absorbance values (A_{405nm}) did not differ significantly ($P < 0.05$) from 1.96 ± 0.25 recorded for *N. tabacum* cv. Harrow Velvet PVY^N-infected control. Four tobacco cultivars were resistant to PVY^N infection: Virgin A Mutante (VAM), NC 744, TN 86, and PBD 6. Virus was not detected on any of the inoculated plants by ELISA or in leaf dips examined under an electron microscope. Plants were identical in appearance to healthy uninoculated controls. Back-inoculation of sap to susceptible *N. tabacum* Harrow Velvet failed to produce infection.

VAM carries a single recessive gene that confers resistance to a variety of PVY strains (2,9). Two of the other resistant cultivars, NC 744 (3) and TN 86 (12), also carry the VAM gene. The breeding background of PBD 6 is unknown. Havana 307, a cultivar that is highly tolerant to the major U.S. strains of PVY and to three other strains from Chile, South Africa, and Hungary (7), also was tolerant to PVY^N, as were two Polish cultivars, Wanda and Wisana. Tolerant lines were characterized as those with mild mosaic and reduced virus titer with or without necrotic symptoms. Absorbance values in ELISA did not differ significantly ($P < 0.05$) from 0.38 ± 0.28 for the three varieties. Tolerant lines were not stunted and were very similar in overall growth to the healthy controls. NCTG51, a backcross derivative of NC602 (15) that was previously found to be tolerant to the necrotic effects of the PVY strain VAM-B and the stunting caused by the NN and Spanish strains, was not resistant to PVY^N. All of the registered Canadian tobacco cultivars were susceptible to PVY^N. ELISA absorbance values did not differ significantly from the infected controls.

Tests of species demonstrated that *N. kawakami* exhibited mild stunting and slightly reduced virus titers with ELISA ($A_{405nm} = 0.89 \pm 0.20$). *N. rustica* var. NRT was tolerant to PVY^N infection, with low virus titers ($A_{405nm} = 0.37 \pm 0.14$) and no visible symptoms. Electron microscopy confirmed the presence of low numbers of virus particles in the sap triturate of NRT. The remaining species, *N. debneyi, N. glutinosa, N. megalosiphon,* Heurch. & Muell. and *N. sylvestris*, exhibited varying levels of stunting, mosaic, mottling, and veinal necrosis with ELISA absorbance values that did not differ significantly ($P < 0.05$) from the infected controls.

Of the somatic hybrids, no resistance or tolerance was found in the *N. debneyi* × *N. tabacum* (HF7A, HF5, HFD21D), *N. rustica* × *N. tabacum* (HDR4, HDR6, HDR14), *N. sylvestris* × *N. tabacum* (HD52, HD525, HD530), or *N. tabacum* × *N. glutinosa* (HDG1, HDG5, HDG7) hybrids, and ELISA absorbance values did not differ significantly from the infected controls.

These results demonstrate sources of genetic resistance to PVY^N in some tobacco cultivars; however, one of the sources, the VAM gene, may find limited application in flue-cured tobacco because of an association with poor quality and increased susceptibility to some insects (13). Sources of tolerance were identified within *N. tabacum*, such as Havana 307, and its introduction should be possible. *N. kawakami* is readily crossed with tobacco, and introgression of tolerance genes from this species also is possible. Although *N. rustica* var. NRT was highly tolerant, the somatic hybrids of this species and

cultivated tobacco were not. It is possible that this tolerance is recessive or multigenic or that the chromosomes carrying the resistance factors were eliminated during hybridization.

LITERATURE CITED

1. Anonymous. 1988. Tobacco production recommendations. Publication 298, Ontario Ministry of Agriculture and Food, Guelph.
2. Burk, L. G., Gooding, G. V., Jr., and Chaplin, J. F. 1982. Reaction of *Nicotiana* species and cultivars or breeding lines of *Nicotiana tabacum* to three strains of potato virus Y. Tob. Sci. 26:86-88.
3. Chaplin, J. F., Burk, L. G., Gooding, G. V., Jr., and Powell, N. T. 1980. Registration of NC744 tobacco germplasm. Crop Sci. 20:677.
4. Clark, M. F., and Adams, A. N. 1977. Characteristics of the microplate method of enzyme-linked immunosorbent assay for the detection of plant viruses. J. Gen. Virol. 34:475-483.
5. Gambourg, O. L., and Wetter, L. R. 1975. Callus and Cell Culture. Pages 1-9 in: Plant Tissue Culture Methods. National Research Council of Canada, Prairie Regional Laboratory, Saskatoon, SK.
6. Gooding, G. V., Jr., and Lapp, N. A. 1980. Distribution, incidence and strains of potato Y in North Carolina. Tob. Sci. 24:200-203.
7. Gooding, G. V., Jr., Wernsman, E. A., and Rufty, R. C. 1985. Reaction of *Nicotiana tabacum* cultivar Havana 307 to potato virus Y, tobacco vein mottling virus, tobacco etch virus and *Peronospora tabacina*. Tob Sci. 29:32-35.
8. Grybauskas, A. P. 1990. Tobacco disease loss evaluation committee report, 1989. Tob. Dis. Council Tob. Workers' Conf. Charleston, SC.
9. Gupton, C. L., and Burk, L. G. 1973. Location of the factor for resistance to potato virus Y on tobacco (*Nicotiana tabacum* L.). J. Hered. 64:289-290.
10. McDonald, J. G., and Kristjanson, G. T. 1993. Properties of strains of potato virus Y^N in North America. Plant Dis. 77:87-89.
11. McDonald, J., Machatchy, I. A., Sheidow, N. W., Watson, M. C., and Reeleder, R. D. 1991. Tobacco veinal necrosis strain of potato virus Y. Fact sheet 181/630. Ontario Ministry of Agriculture and Food, Guelph.
12. Reddick, B. B., Miller, R. D., and Gooding, G. V., Jr. 1991. Resistance in *Nicotiana tabacum* cultivar TN86 to naturally occurring tobacco viruses. Tob. Sci. 35:96-99.
13. Smeeton, B. W. 1976. Budworm susceptibility of PVY resistant cultivars. Tob. Sci. 20:156.
14. Sproule, A., Donaldson, P., Dijak, M., Bevis, E., Pandeya, R., Keller, W. A., and Gleddie, S. 1991. Fertile somatic hybrids between transgenic *N. tabacum* and transgenic *N. debneyi* selected by dual antibiotic resistance. Theor. Appl. Genet. 82:450-456.
15. Witherspoon, W. D., Jr., Wernsman, E. A., Gooding, G. V., Jr., and Rufty, R. C. 1991. Characterization of a gametoclonal variant controlling virus resistance in tobacco. Theor. Appl. Genet. 81:1-5.