Turnip Mosaic Virus in the Illinois Horseradish Germ Plasm Collection

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ABSTRACT

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In fall 1982, turnip mosaic virus (TuMV) was detected by enzyme-linked immunosorbent assay (ELISA) in 92% (136/148) of the horseradish clones in the Illinois germ plasm collection. Sap extraction from mature symptomatic leaves was more sensitive than leaf disks for detecting TuMV by ELISA. TuMV was detected by symptomatology and by ELISA in the highest percentage of clones during cool spring or fall weather. No horseradish clone remained both symptomless and negative by ELISA throughout the 2 yr of this study.

Horseradish (*Armoracia rusticana* Gaertn., Mey., & Scherb.), a hardy perennial herb in the Brassicaceae, is grown commercially in temperate regions. Illinois is the leading producer of horseradish in the United States, and the University of Illinois has maintained an extensive collection of horseradish genotypes as part of its breeding program to increase yields and disease resistance.

Turnip mosaic virus (TuMV) is one of the most common pathogens of horseradish. It is transmitted by several species of aphids and may also be transmitted during preparation of secondary root stocks for vegetative propagation. Many authors have stated that virtually all horseradish plants appear to be infected with TuMV (7,8,11,13,15). Symptoms of TuMV on horseradish include chlorotic mosaic or ring spots on leaf blades, dark streaks on petioles, and rarely, vein-clearing. Symptoms are most severe when plants are grown at cool temperatures (≤16 C) and are often masked at warm temperatures (28 C) (12). The host range and physical properties of several TuMV strains found in horseradish have been determined (1,6,11). Other viruses found in horseradish include arabis mosaic virus and cauliflower mosaic virus (4,5,15).

Several attempts have been made to free horseradish from virus by heat treatment or tissue culture (5,7,10). Tissue culture methods have been successful; however, once in the field, most plants become reinfected within one growing season. Therefore, development of cultivars resistant to TuMV seems the best means of control.

Three surveys of horseradish germ plasm collections for virus infection have been conducted (1,5,15). In each case, some clones were found in which no virus could be detected, suggesting that cultivars resistant to TuMV may exist. Our study was undertaken to determine if there are any horseradish clones resistant to TuMV in the Illinois germ plasm collection. Such clones could be the basis of a horseradish breeding program for resistance to TuMV.

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MATERIALS AND METHODS

The horseradish germ plasm collection at the Illinois Agricultural Experiment Station (AES) consists of 47 cultivars from 11 European countries, 15 cultivars from North America, and 86 cultivars developed in the Illinois AES horseradish breeding program. Two methods were used to detect TuMV infection of horseradish plants in the germ plasm collection: symptomatology and the enzyme-linked immunosorbent assay (ELISA).

The TuMV strain used for antiserum production was isolated from an infected horseradish plant collected near East St. Louis, IL. The virus was passaged through turnip (*Brassica napa* L.) and purified from mustard (*B. pereridis* L. 'Tendergreen') (3).

The gamma globulin (IgG) fraction of TuMV antiserum (titer = 1,024) was purified by precipitation with ammonium sulfate and passage through a DE-22 column (Bio-Rad Chemical Division, Richmond, CA). Immulon microtiter plates (Dynatech Laboratories, Inc., Alexandria, VA) were coated with 200 μl of IgG at 4 μg/ml for 12 hr at 4 C. Between each step, plates were rinsed seven times with phosphate-buffered saline containing 0.05% Tween 20 (PBS-T) (2). Samples were incubated overnight at 4 C. Two hundred microliters per well of IgG conjugated to alkaline phosphatase (diluted 1:200) were incubated for 4 hr at room temperature. Substrate (*p*-nitrophenyl phosphate, Sigma Chemical Co., St. Louis, MO) (200 μl/well at 1 mg/ml) was incubated for 45 min at room temperature, then 50 μl of 3 M NaOH was added to each well to stop the reaction. Samples were diluted 1:5 in water filtered in a Milli-Q water purification system (Millipore Corp., Bedford, MA), and absorbances at 405 nm were read in a Beckman DU spectrophotometer (Beckman Instruments, Palo Alto, CA). Samples with A_{405}

readings greater than four standard deviations above the mean of six samples from healthy horseradish were considered positive (9).

Different parts of horseradish plants known to be infected with TuMV were tested by ELISA to determine which part was most consistently positive. Roots, young leaves, and fully expanded leaves from five plants with mosaic or ring spot symptoms and leaves without symptoms were tested. Tissue samples (1 g) were ground in 4 ml of PBS with a mortar and pestle and 200 μl of the expressed sap was added to an ELISA well. Because TuMV is not seed-transmitted, in all ELISA tests samples from horseradish plants raised from seed were used as negative controls.

Because sample grinding was time-consuming, the sensitivities of the sap extraction and leaf-disk sampling (14) methods were compared using samples from horseradish plants with mosaic symptoms in June and July 1982. One gram of leaf tissue ground in 2 ml of PBS was compared with five leaf disks per well.

Horseradish in the germ plasm collection was tested by ELISA in July 1982, when plants had few symptoms, and again in September, when symptoms were strongly expressed. An attempt was made to sample leaves displaying all symptom types observed in a clone. A single sap extract was made from all leaves chosen from each cultivar. At the sampling times, clones were rated symptomatic or symptomless. In addition, symptoms on all clones were rated in October 1981 and May and June 1982. Four horseradish clones were retested for TuMV by ELISA in September 1983.

RESULTS

All samples from known healthy horseradish seedlings or mature plants freed of virus by tissue culture were negative for TuMV by ELISA. In preliminary tests of infected horseradish plants, samples from mature leaves with symptoms gave the most and the highest positive ELISA readings compared with samples from roots, young leaves, and symptomless mature leaves. This was particularly true for plants with mosaic symptoms where samples from symptomatic, fully expanded leaves were always positive. In contrast, samples from symptomatic leaves of plants showing ring spots were sometimes negative. Therefore, all samples taken from the horseradish germ plasm collection were mature leaves.

In samples collected in early June, when symptoms were strongly expressed, TuMV was detected by ELISA in leaf disks. In July, however, when symptoms were masked, virus was not detected in disk samples although sap samples from the same leaves were positive. Therefore, to maximize TuMV detection, germ plasm collection samples were ground to express sap for ELISA.

When the horseradish germ plasm collection was sampled in July, only 60% of the clones had distinct TuMV symptoms, and fewer than half of these were positive by ELISA. All plants with no symptoms were negative. In September, nearly all clones had TuMV symptoms (Table 1), and very few symptomatic plants were negative by ELISA. Some symptomless cultivars were positive by ELISA and some were negative (Table 1). Only four cultivars were both symptomless and negative by ELISA in fall 1982. All four cultivars had ring spot symptoms in spring 1982, and two were positive for TuMV by ELISA at other testing times.

Table 1. Correlation of turnip mosaic virus (TuMV) symptoms with enzyme-linked immunosorbent assay (ELISA) readings for samples taken from the Illinois horseradish germ plasm collection in fall 1982

TuMV symptoms[a]	ELISA readings[b]	Number of cultivars
+	+	130
+	−	8
−	+	6
−	−	4
Total	···	148

[a] Plants with mosaic or ring spot symptoms were rated positive.

[b] ELISA values greater than four standard deviations above the mean of six healthy horseradish samples were considered positive (9).

DISCUSSION

Turnip mosaic virus was detected by ELISA in 92% of the horseradish clones in the Illinois AES germ plasm collection. This detection level is considerably higher than those reported in previous surveys of the crop. In 1968, Hickman and Varma (5) determined on the basis of symptomatology, electron microscopy, and serology that 36% of 47 clones (most from Europe) were infected with TuMV. Using similar methods, Shulka and Schmelzer (15) (1972) in West Germany detected TuMV in 41% of 120 horseradish plants. In 1959, Chenulu (1) tested 72 horseradish clones in the Illinois AES collection by local lesion assay to *Chenopodium amaranticolor* and found 73% infected with TuMV. Plants with low TuMV titer were probably missed in these studies, particularly because horseradish contains inhibitors to mechanical transmission (15). Unfortunately, none of the clones that were negative in Chenulu's test were still present in the Illinois collection in 1982.

ELISA is a rapid, highly sensitive tool for screening germ plasm for viruses. No false positive reactions were noted from horseradish samples tested by ELISA; however, the time of year when plants were sampled greatly influenced the detection of TuMV. Virus titer is much lower in warm weather than in cool and may drop below the level detectable by ELISA. It was therefore important to screen germ plasm during spring or fall.

The best method for sampling horseradish plants for ELISA was extraction of sap from mature leaves. Other plant parts gave lower or negative readings, and disk samples were not as sensitive as sap samples. However, samples taken from mature leaves of infected horseradish plants with ring spot symptoms were not always positive by this method. This problem might be due to uneven distribution of virus in these tissues, low concentration of virus in the sample, or the presence of a different TuMV strain. The specificity of TuMV strains in ELISA has not been examined.

Of the 148 clones sampled in fall 1982, only four were both symptomless and negative by ELISA. The other six symptomless plants were positive by ELISA. Since healthy horseradish plants never gave false positive reactions, these six plants were probably symptomless carriers of TuMV. A few symptomatic plants were negative by ELISA. Symptoms of TuMV infection are not easily confused with environmental stress, so these plants were probably infected with TuMV below detectable levels or with another TuMV strain.

Of the four symptomless, negative clones found in fall 1982, one (829a) had symptoms in spring 1982 and was positive by ELISA; the other three also had ring spot symptoms in early June 1982 but were not tested by ELISA at that time. In fall 1983, clone 842a was positive by ELISA, but the other two clones (595a and 635a2) were negative. Thus, no clone of the 148 remained both symptomless and negative by ELISA throughout this study.

It does not appear likely that any horseradish clones are resistant to TuMV. Many cultivars do not seem weakened by the presence of the virus, and most commercial fields are planted with cultivars that display marked symptoms of virus infection. In recent years, increased resistance to TuMV has been a goal in the horseradish breeding program at the University of Illinois. The potential usefulness of clones in which virus is not detectable by ELISA in a breeding program for resistance to TuMV should be examined.

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