Seed Transmission of Maize Chlorotic Mottle Virus

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ABSTRACT

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Seed corn (*Zea mays*) produced in commercial nurseries in Hawaii was harvested from symptomatic plants naturally infected with maize chlorotic mottle virus (MCMV). In Nebraska greenhouse studies, the seeds were planted and 14-day-old seedlings were assayed for MCMV. Seed transmission was found by ELISA and confirmed by symptomatology and mechanical transmission in 17 of 42,000 plants from 25 seed lots submitted by three seed companies. Some of the factors relating to seed transmission were tested.

Maize chlorotic mottle virus (MCMV) was first described from Peru in 1974 (3) and soon thereafter was reported from corn (*Zea mays* L.) in the Republican River valley in Kansas (13) and Nebraska (5). In 1989 it was reported in Mexico (2), and during the winter of 1989–1990 it was found in winter seed production nurseries and domestic sweet corn fields on the Hawaiian island of Kauai (8,9,14).

Several species of beetles are vectors for MCMV (12) but they are not known to move the virus over long distances (7). Previous studies have not shown seed transmission of MCMV in several species of plants, including corn (1). Therefore, the mechanism for long-distance dissemination of this virus remained unknown. The study reported in this paper was undertaken to better understand the potential for seed transmission of MCMV and its possible role in virus spread.

MATERIALS AND METHODS

Seeds were harvested from corn grown in Hawaii that was naturally infected with MCMV. Seed lots, chosen to represent a wide range of germ plasm diversity, were contributed by three different seed companies. Twenty-five seed lots were tested, some of which differed only in the time or method of harvest. All seed lots were dried to about 15% moisture after harvest.

In Nebraska, the seeds were planted in a steamed mixture of soil, sand, and vermiculite in sterilized galvanized metal trays, 35 × 50 cm. Each tray contained five rows with 20 seeds per row. Representative stand counts indicated more than 90% germination. Seedlings were grown in a greenhouse, between mid-March and mid-May, without supplemental light at a temperature of 25–30 C. Seedlings emerged in 4 days and by 14 days were in the four- to five-leaf stage. Stringent measures of isolation, confinment, handling, and insect control were taken to avoid accidental infections.

Standard double antibody sandwich (DAS) enzyme-linked immunosorbent assay (ELISA) procedures were followed in the assay for infected seedlings (4). The antisera, which had been raised in rabbits against a Nebraska isolate of MCMV, reacted strongly with virus samples from Hawaii but not with healthy plant sap. In two preliminary ELISA dilution series tests, using a Hawaiian isolate of MCMV, we found that the virus was easily detectable in homogenates of infected leaves at dilutions of 1:4,000, w/v (ELISA OD >0.9).

Fourteen days after seeding, the terminal 5–8 cm of the youngest leaf was harvested from each plant in a row. All harvested leaves from a row were combined into a single sample of approximately 3 g and homogenized in 15 ml of 0.01 M $NaPO_4$ buffer pH 7.0. The homogenate was filtered through cheesecloth, and 0.1 ml was used for ELISA; dilution of tissue was approximately 1:5, w/v. In each daily sampling, known positive and negative controls and blind controls unknown to the person conducting the ELISA were included. All controls were the Nebraska isolate of MCMV. All samples were run in duplicate or triplicate.

Plants of composite samples yielding positive reactions by ELISA were examined individually, and in every case symptoms were detected on one plant within the row. Leaf samples from that plant were tested by ELISA and by infectivity to corn for confirmation of MCMV infection.

At the conclusion of the first screening, we observed that the three seed lots with the highest level of seed transmission had been shipped in their entirety and contained many more discolored, small, cracked, or broken seeds than the other lots. To determine whether the poor-quality seeds were the carriers of MCMV, remnant seed from seed lot 7 were hand-sorted into three classes: 1) large, bright, plump, and good quality; 2) small or discolored but otherwise sound; and 3) immature, chaffy, shriveled, or damaged. These seeds were planted and tested in the manner described.

Two experiments were conducted to determine whether virus transmissions originated from within the seed or through the roots by surface contamination. In the first experiment, greenhouse-grown Golden Cross Bantam sweet corn in the three- to four-leaf stage was carefully dislodged from the pot and the exposed roots were inoculated with 1/1,000 or 1/100 dilutions of purified virus. Leaf inoculations of similar plants served as controls. Each treatment consisted of three pots with five plants each.

In the second experiment, to test for root infection from plant debris, the seed furrows of three trays were lined with chaff from infected ears and planted with Golden Cross Bantam sweet corn seed.

RESULTS

Of 42,000 seeds from 25 seed lots, 17 transmitted MCMV to seedlings: 1 of 3,000 seeds in lot 5, 10 of 3,000 seeds in lot 7, 2 of 3,000 seeds in lot 8, and 4 of 2,000 seeds in lot 9. Every case was confirmed by ELISA and by mechanical transmission. Seed transmission was not random—16 of the 17 MCMV-positive transmissions came from 8,000 seeds (lots 7–9) submitted by one seed company.

When we tested the remnant seed from seed lot 7 to determine if seed quality and the amount of seed transmission were related, we found three MCMV-positive plants from 1,580 class 1 (good) seed, no infected plants from 320 class 2 seed, and one MCMV-positive seedling from 71 class 3 seed. Although this test was conducted 2 mo after the initial evaluation of this seed lot, there was no apparent loss of seed transmission, and there was no correlation between seed quality and seed transmission.

In the tests of root vs. leaf inoculation with an inoculum dilution of 1/1,000, it took 5 days for symptoms to appear on the leaf-inoculated plants but 30 days for

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the first leaf symptoms to appear on root-inoculated plants. With an inoculum dilution of 1/100, symptoms appeared on the leaf-inoculated plants in 5 days and on the root-inoculated plants in 13 days.

None of the 300 seeds planted into the chaff from infected ears developed symptoms by 30 days after planting.

DISCUSSION

Seed transmission of MCMV appears to be similar to that of maize dwarf mosaic virus (MDMV). Most tests with MDMV have demonstrated very low levels of seed transmission, i.e., one of 22,189 seeds (11), one of 11,448 seeds (6), and two of 29,735 seeds (18). Shepherd and Holdeman (16) found 17 of 9,485 plants infected, but 14 of the positive transmissions came from one lot of 3,163 seedlings.

In view of the results of this study, it is not surprising that Bockelman et al (1) were unable to demonstrate seed transmission of MCMV. Seed transmission of this virus, as of MDMV, bean pod mottle virus (10), and wheat streak mosaic virus (6), seems to be rare, occurring only in certain seed lots and controlled by unknown factors.

We are confident, because of the rapid development of symptoms and the stringent experimental controls, that the virus infection came through the seed rather than by other means. Some of the seedlings had symptoms on every leaf, and all infected seedlings had symptoms on all leaves above the second leaf. Very early infection through external contamination of the roots could not be demonstrated experimentally by growing seedlings in infected plant debris. Therefore, accidental transmission in the greenhouse would have had to occur on aboveground parts and symptoms would not have developed as rapidly as was observed in this study.

The slow development of leaf symptoms after root inoculations in greenhouse tests corresponds to disease development in infested fields of the Republican River valley in Kansas and Nebraska. In this area, a soil-inhabiting organism may be involved in infection, because MCMV overwinters only in previously infested fields (5,7,15,17). Infection of seedlings in the spring is suspected to start in the roots, and symptoms are slow to develop. In Hawaii, where there is continuous corn culture, epidemiological factors appear to be different (8,9,14). There, the virus and vector increase in older plants and move to young corn seedlings, where symptoms appear early and are more severe.

In every composite sample with an MCMV-positive test by ELISA, we found a symptomatic plant in the positive row. That plant later proved positive by a second ELISA and by mechanical transmission. Through the course of this study we found several plants with viruslike symptoms but which were not MCMV-positive in the original ELISA screening. To be certain that these plants were not false negatives, several were individually tested for MCMV by ELISA or by mechanical transmission or were held for further symptom development. In every case, test results for these suspicious plants were negative and the plants eventually recovered.

The seed lots that gave the high transmission rates were harvested from plants that were uneven in maturity and, therefore, may have contained a few immature kernels. A test of remnant seed showed that plump, bright, healthy-appearing seed transmitted virus. Seed moisture levels, test weight, and other indications of maturity were normal for good-quality seed. These values were based on bulk seed and not on individual kernels, however. Individual seeds may have varied in moisture level or maturity. Storing the remnant seed of lot 7 for 2 mo at room temperature should have allowed the seed to reach a uniform state of maturity and moisture content without altering the seed transmission rate.

From this study we conclude that MCMV can be introduced into corn production areas through seed transmission. It is possible, but unlikely, that the virus was introduced into Hawaii through infected seed. Seed planted in Hawaii did not originate from MCMV-infested areas. Producers in Kauai suspect that the virus may have been present in trace amounts during past years but became apparent only when heavy rains prevented insect control and thus favored the epidemic during 1989–1990 (8,9,14).

We do not know the geographic origin of the MCMV in Hawaii, but we are currently attempting to characterize MCMV isolates from many localities. These experiments may help us understand the dispersion of the virus.

We do know that seed transmission makes MCMV a threat to the seed corn industry in Hawaii and elsewhere. The three companies involved have destroyed all seed corn produced on Kauai during this season. In an effort to control the disease, there has been a 4-mo period in which no corn has been grown.

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