A Novel Way to Transmit Plant Viruses

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SUMMARY

Plants were infected with viruses by electro-endosmosis. This was demonstrated with three viruses, one being maize streak virus (MSV) which, to date, has been transmissible only by leaf hoppers.

In a previous communication Polson (1977) reported that tobacco mosaic virus particles could be extracted from an infected tobacco leaf by establishing a potential gradient of approx. 3 V/cm parallel to the midrib of the leaf, the tip of which had been cut off. Electro-extraction was done for 24 h into 10 mM-borate buffer, pH 8.6. As virus particles could be extracted from the tissue of plants using an electrical potential gradient, it appeared logical that the process could be reversed and that virus particles could be caused to migrate into the leaf tissue and give rise to infection.

Some experiments on the effect of a direct current on plants showed that the movement of fluid into plants through their roots may be effected by passing an electric current from the roots to the stem. Contact with the plant was made by insertion of a platinum wire into the stem at the highest possible position. The polarity was of vital importance. For example, young (10 cm) mustard plants (Brassica nigra cv. White London) could be dehydrated by applying a gradient of 1 V/cm overnight with the stem attached to the negative electrode, the positive being placed in the soil close to the roots. In plants in which the polarity was reversed, no dehydration occurred except in the leaves above the positions where the platinum electrodes were inserted. These leaves were dry after 48 h; obviously the movement of the fluid into these regions had stopped or had been reversed. A direct current with approximately the same potential gradient had similar effects on bean plants (Phaseolus vulgaris cv. van Zyls).

In other experiments the dehydration was counteracted by slicing off the tips of bean plants, immersing the damaged leaves into 10 mM-phosphate buffer, pH 7.5 and passing a current at approx. 1 V/cm through the plants for a period of 24 h.

In the first instance, the soil in the pots was connected to the battery’s positive pole and the buffer to the negative and in the second the polarities were reversed. No dehydration of the plants occurred, indicating that fluid had moved from the soil through the plant system to the buffer in the Petri dish and in the other instance, in the reverse direction. The experiments were conducted in the laboratory at approx. 21 °C, no precautions being taken to minimize transpiration as no wilting occurred.

From these observations it was concluded that fluid could be moved into and out of plants by electro-endosmosis. Using the electro-endosmotic flow of virus-containing fluid we have attempted to infect young wheat plants with brome grass mosaic virus (BMV), Beth strain (an isolate from natural grasses in the Orange Free State, South Africa), young broad bean plants (Vicia faba cv. acquadulce) with an unidentified seed-transmitted broad bean virus which had filamentous particles approx. 700 nm long (isolated by Mrs J. Barber of our department) and young maize plants with maize streak virus (MSV). We included MSV in the series of experiments as this virus has so far only been transmitted by the leaf hopper Cicadulina mbila.

In the electro-infection experiments 9 V dry cell batteries were used. The experimental arrangement is shown diagrammatically in Fig. 1. Electrical leads were soldered to the
battery poles and short lengths of platinum wire were attached to the distal ends of the insulated leads. One platinum wire was pushed into the soil close to the stem of each plant and the other was submerged in buffer in the electrode vessel. The buffer was connected via a filter paper wick with a suspension of the virus particles contained in a small Petri dish and the cut or injured leaves of the test plants held in the virus suspension. Thus an electrical circuit was made through the soil, plant, virus suspension and buffer in the electrode vessel.

In every experiment four pots with plants were used except in the experiment with maize. Broad beans were grown as single plants in pots, maize as three or four and wheat as a minimum of 20 plants/pot. One broad bean plant was infected mechanically with the seed-transmitted virus, a leaf from a second plant was sliced and the injured part held in the inoculum in 10 mM-sodium phosphate buffer, pH 7.5, but no current was passed through the plant. The injured leaf of a third plant was submerged in the virus suspension which was connected via the wick to the negative pole of the battery, the soil in the pot being connected to the positive pole (Fig. 1); the fourth was arranged like the third but with the polarity reversed.

In the experiment with the maize plants and MSV no effort was made to infect the plants mechanically. Attempts at electro-infecting the plants were conducted in the same manner as with the broad beans using 10 mM-sodium phosphate, pH 7.5. One plant in three in each pot was treated, the remaining two serving as controls.

The wheat plants were approx. 12 cm high. The plants in one pot were mechanically inoculated with BMV. Four plants in each of the remaining pots were loosely held together with elastic bands, their tips sliced and immediately immersed in the BMV inoculum contained in 20 mM-acetate buffer, pH 5.4. No electric current was passed through plants in one pot. Groups of four plants in the remaining two pots were treated in a similar way to the broad beans, the only difference being that four plants were treated as a unit. Acetate buffer (20 mM, pH 5.4) was used in the experiments as it was known that BMV particles swell in buffers of neutral and higher pH (Bockstaler & Kaesberg, 1962) and were consequently less viable.

In all experiments the concentration of virus in the Petri dishes was 1 mg/ml or less; the viruses were partially purified by ultracentrifugation of clarified sap at 100,000 g for 90 min, followed by resuspension of the pellets in the appropriate buffer. The potential gradient of approx. 1 V/cm was maintained through the plants for 24 h, then the leaves, which had been in contact with the inoculum, were rinsed and the plants placed in a growth room where they were examined daily for the appearance of virus symptoms.
Passing the current through the plants at 1 V/cm caused no wilting which indicated that the fluid in the plant tissue which was transported by electro-endosmosis from the root system was replaced by the fluid of the inoculum in the Petri dishes. Voltage gradients of approx. 3 V/cm caused blackening of the stems of broad bean plants in 24 h and were therefore not used in the electro-infection experiments.

In each of two experiments wheat plants mechanically inoculated with BMV showed systemic symptoms after 2 days. The plants whose injured leaves were dipped in the inoculum showed no sign of infection after 14 days. The ‘electro-inoculated’ plants which had their roots connected to the positive pole showed systemic symptoms in all four plants after 7 days, but no symptoms were shown after 14 days by the plants which had their roots connected to the negative pole of the battery. Sap from the plants which showed symptoms of BMV reacted positively in Ouchterlony double-diffusion serological tests, whereas sap from plants which showed no symptoms of virus infection did not react with immune serum to BMV. The isoelectric point of BMV is approximately at neutral pH (Bockstaler & Kaesberg, 1962). At pH 5.4 the virus particles were positively charged and should have migrated to the negative pole, yet the migration was to the positive. The obvious explanation for this apparent ambiguous effect was that the electro-endosmotic flow into the plant tissue exceeded the reverse migration of the BMV particles, thus causing them to move into the plant. The sparring of the precipitin band which was observed may be due to changes which occurred in the virus particles during the 5 days between appearance of symptoms and harvesting (see above).

In the two experiments with the seed-transmitted broad bean virus, done in the same way as BMV and wheat plants but using 10 mM-sodium phosphate buffer, pH 7.5, the only plants which showed systemic symptoms were those which were mechanically inoculated and those whose roots were connected to the positive pole of the battery. The plants which were mechanically inoculated showed disease symptoms after 8 days and those subjected to electro-infection after 11 days. When the sap from these plants was examined in an electron microscope, using the leaf dipping technique, filamentous particles were only found in those plants which showed symptoms.

In the experiment with MSV, the young maize plant whose roots were connected to the positive battery pole for 24 h showed typical systemic symptoms of MSV infection after 3 weeks, whereas none of the remaining plants in the pot nor the plant whose roots were connected to the negative pole or the plant whose injured leaf tip was immersed in the inoculum for 24 h without current, showed any MSV disease symptoms. As no immune serum to MSV particles was available no serological experiments confirming the presence of MSV in the plant sap could be made.

The experiments reported here show that electro-infection is an additional means of infecting plants with viruses. The advantage this method has over conventional methods is that the infection may be established with very low concentrations of virus particles and, more importantly, that some viruses, such as MSV, which are normally only transmitted by vectors, may possibly be experimentally transmitted to susceptible plants.

Department of Microbiology
University of Cape Town
Rondebosch 7700
Republic of South Africa

A. POLSON
M. BARBARA VON WECHMAR

REFERENCES


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