Virus Indexing of Callus Cultures of Chrysanthemum morifolium by Tissue Implantation and Mechanical Inoculation

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ABSTRACT

The successful use of plants derived from callus tissue cultures originating from meristem-tips for rapid propagation of chrysanthemums is dependent upon reliable indexing of the callus for freedom from virus diseases. Implantation of callus tissue into indicator cultivars of chrysanthemums by means of surgical cannulas has proved to be a rapid and efficient indexing technique for chrysanthemum stunt, chrysanthemum mosaic, and chrysanthemum chlorotic mottle. Mechanical inoculation of leaves of *Nicotiana tabacum* 'Samsun' with sap extracted from infected callus tissue has proved to be rapid and reliable for indexing for the chrysanthemum strain of tomato aspermy virus.

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The possibility of clonal propagation of chrysanthemums (*Chrysanthemum morifolium* Ramat.) by means of plants derived from callus developing from meristem-tips has been described by Ben-Jaacov & Langhans (1). Success of such a technique, as with any form of clonal propagation, would be dependent, among other things, upon reliable determination of freedom from known viruses in the callus source of the plants. This paper describes methods that have proved effective for indexing chrysanthemum callus for chrysanthemum stunt, mosaic, aspermy, and chlorotic mottle viruses (3, 8, 10, 11).

MATERIALS AND METHODS.–Cuttings were obtained from chrysanthemum cultivars known to contain chrysanthemum stunt, chrysanthemum mosaic, and a chrysanthemum strain of tomato aspermy. Each cutting was divided into three segments: a = the meristem-tip; b = the 5-cm segment below it; and 3 = an internodal stem segment 0.5 cm long, herein called the shoot base, taken just below segment b. Segments a and b, after being surface sterilized (5 min in 15% Clorox followed by rinsing in sterile water), were placed on a semisolid Murishige & Skoog (9) medium containing 10% coconut milk and 2 ppm naphthalene acetic acid, where they formed large amounts of callus. The b segments were rooted, and the resulting plants used as controls. After 2 months, the virus tests were initiated.

Two methods were tested for stunt and mosaic viruses; (i) grafting of a virus-free (2, 7) scion of an indicator cultivar onto the callus being tested; and (ii) implanting infected callus tissue into virus-free indicator plants. Although grafts of type i in the present tests were made successfully, the technique was found to be difficult and was discontinued.

The fact that viruses can be transmitted by implantation of stem, leaf, sepal, stamen, or fruit tissue (5) suggested the possibility of transmission of viruses by implantation of callus tissue. Successful callus-grafting or implantation into stems of normal plants has been reported (6). The use of the surgical cannula technique (4) has made such implantation simple and fast. In the present tests, a plug removed with the cannula from the callus tissue under test was inserted in the hole in the indicator plant and covered with parafilm, no other protection being needed. A conventional splice graft of an indicator scion onto the plant developing from segment b was used as a control. The grafted plants were covered with plastic bags to prevent rapid drying.

The chrysanthemum stunt source for development of infected callus was a stunt-infected plant of the cultivar Fanfare, and the indicators were plants or scions of the cultivar Blanche infected with Noordam's B virus (Keller's virus Q). The chrysanthemum mosaic source was a mosaic-infected plant of the cultivar Fanfare, and the indicators were virus-free plants of the same cultivar. The specific mosaic, which produced severe leaf distortion in Fanfare, was not identified.

Mechanical inoculation tests were employed for transmission of aspermy virus, using an aspermy-infected plant of the cultivar Giant Betsy Ross as the source of infected callus or leaf tissue. Sap extracted from infected callus tissue was tested, and sap from infected leaf tissue was used as the control. The callus or leaf tissues were ground in sodium sulfite buffer (5 g/liter) with Carborundum powder, and the extracts were rubbed on leaves of tobacco seedlings (*Nicotiana tabacum* 'Samsun').

RESULTS AND DISCUSSION.–*Stunt*.–Callus implantation was as effective in transmitting stunt as was conventional splice grafting. Transmission was 100% effective with both techniques: six of six grafted plants and 12 of 12 callus tissue implanted indicator plants developed distinctive stunt symptoms.

Mosaic.–Transmission of the Fanfare mosaic virus by callus implantation was less reliable than transmission of stunt. Only three of four plants implanted with meristem callus tissue and three of six implanted with shoot base callus tissue developed symptoms. Presence of mosaic in the source plants was confirmed, as plants developing from the b segments in the four test plants used were all positive. Less efficient transmission of the Fanfare mosaic by

cannula implantation of infected stem tissue was also noted by Dimock et al. (4).

Aspermy.—The use of sap extracted from aspermy-infected callus tissue for mechanical transmission of this virus was as reliable as the use of sap extracted from infected leaves. Eleven of 16 tobacco plants inoculated with sap from infected callus tissue developed characteristic symptoms, as did five of eight plants inoculated with sap from infected leaves.

The three viruses tested were transmitted successfully from infected callus to indicator plants, stunt and mosaic from callus to indicator plants by tissue implantation, aspermy by mechanical inoculation of tobacco test plants with sap from infected chrysanthemum callus tissue. Limited tests have indicated that chrysanthemum chlorotic mottle virus also may be reliably transmitted by implantation of infected callus tissue into indicator plants. These techniques thus may be employed for reliable indexing of callus tissue employed in the method recently proposed by Ben-Jaacov & Langhans (1) for rapid propagation of chrysanthemums by meristem-tip proliferation.

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