Use of Tissue Culture Potato Plantlets for Investigations of Diseases of Subterranean Plant Parts

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ABSTRACT

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Potato tissue culture plantlets grown in culture tubes were successfully used to establish and monitor disease development in roots and other subterranean plant parts. Host tissue responses to infection and other disease processes were easily observed and recorded permanently (photographically with or without the aid of a microscope) in a nondestructive manner. Use of this technique for pathogenicity and other investigations is discussed.

Observation of the infection and post-infection processes is an integral part of research activities on plant responses to pathogens. This is also true for pathogen–pathogen interactions when investigating diseases caused by a "complex" of pathogens. Repeatable, nondestructive observations of pathogen–host interactions for diseases of subterranean plant parts (roots, stems, etc.) are difficult in field, greenhouse, or laboratory situations. Various techniques are currently used to enable in situ observations of infection and disease-development processes. These techniques include sand, vermiculite, or hydroponic culture media (1,2) and the use of moist paper and transparent glass or plastic chambers (6,8). However, these methods often involve destructive sampling or restricted use of microscopic and photographic tools.

This report describes a simple and rapid technique that enables repeated microscopic observations and photographic recording of disease development.

MATERIALS AND METHODS

Three-week-old potato (*Solanum tuberosum* L.) tissue culture plantlets were grown under sterile conditions on a mineral and vitamin medium (7) containing 8 g/L of agar and 25 g/L of sucrose in culture tubes (1.6 × 22.4 cm) with plastic caps secured with Parafilm M. The plantlets were, depending on the cultivar, approximately 35 mm in height, with three to five leaves and obvious (2–3 mm) root development.

Colletotrichum coccodes (Wallr.) S.J. Hughes, *Verticillium albo-atrum* Reinke & Berthier, *V. dahliae* Kleb., and *Streptomyces scabies* (Thaxt.) Waksman & Henrici were used as typical soilborne pathogens of potatoes. Isolates of the pathogens to be tested had recently been obtained from diseased plant tissues. Pure, 30-day-old cultures grown on either potato-dextrose agar or malt agar (9-cm plastic petri dishes) were cut into 1 × 1 m blocks, and the blocks were used to inoculate the potato plantlets growing in culture tubes.

Under sterile conditions, the inoculum blocks were transferred to the surface of the plantlet agar at the midpoint between the culture tube wall and the plantlet stem. A channel was made with a sterile needle through the plantlet growth medium, from the base of the agar block to the roots of the plantlet. Noninoculated agar blocks were used in control tubes, and other tubes remained noninoculated. Following inoculation, plantlets were incubated at 25 C and 12-hr light/dark regimes (fluorescent, cool-white, 40 W) in controlled-climate chambers.

Five different experiments (two with Bintje and one each with Kennebec, Mirka, and Russet Burbank cultivars) were conducted with three to five replicate culture tubes per pathogenic isolate. For each pathogen species, two to 13 isolates were used. Weekly observations of pathogen and plantlet growth characteristics were made for about 75 days. A dissecting microscope (×100) fitted with a 35-mm camera was used to observe and record host and pathogen responses such as plant height and vigor, leaf size and color, root length and

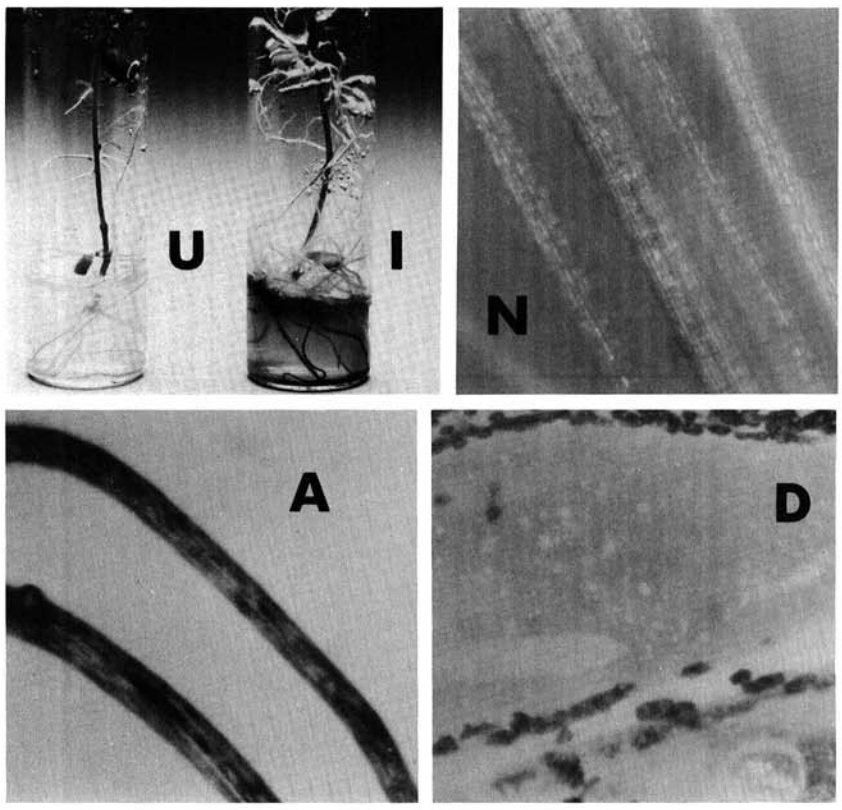

Fig. 1. Potato tissue culture plantlets uninoculated (U) and inoculated (I) with *Verticillium* species, and plantlet roots noninfected (N) and infected with dark mycelium of *V. albo-atrum* (A) and microsclerotia of *V. dahliae* (D).

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Table 1. Potato plantlet growth responses 65 days after inoculation with one of several soilborne pathogens

Pathogen	Plantlet height (mm)	Leaf and symptoms	True roots[a]		Quantity of aerial roots
			Quantity	Lesions	
No treatment	74 ± 4	Healthy	3.0 ± 0	0 ± 0	1.0 ± 0
Agar block	64 ± 7	Healthy	3.0 ± 0	0 ± 0	1.0 ± 0
Colletotrichum coccodes	36 ± 7	Severe necrosis	2.0 ± 0	1.8 ± 0.4	0 ± 0
Verticillium albo-atrum	108 ± 27	Some chlorosis	2.4 ± 0.5	3.0 ± 0	3.0 ± 0
Verticillium dahliae	123 ± 7	Moderate chlorosis	1.8 ± 0.4	3.0 ± 0	2.3 ± 0.4
Streptomyces scabies	57 ± 2	Healthy	3.0 ± 0	1.3 ± 0.4	1.5 ± 0.5

[a] Root quantity and lesion ratings: 0 = none, 1 = few (1–5), 2 = some (6–10), and 3 = many (>10).

numbers, pathogen penetration, root tissue discoloration, pathogen signs, and host symptoms.

RESULTS AND DISCUSSION

Depending on pathogen and cultivar, root contact by the pathogens and initial host responses were observed within 5–10 days after inoculation. Host responses and pathogen growth could be observed for at least 70 days following inoculation, because the large (22.4 cm) tubes accommodated plant growth for this period of time. Plant-growth responses and disease symptoms were observed with the unaided eye during the course of the study (Fig. 1). These included root infections, reduced root growth, stem elongation, leaf and stem chlorosis and necrosis, and formation of "aerial" roots (Table 1). These responses varied somewhat with the pathogen; *Verticillium* species caused typical chlorosis and wilt symptoms, while *S. scabies* and *C. coccodes* caused root lesions and root pruning, respectively. In addition, with the aid of a dissecting microscope, development of various pathogen structures on or within invaded host tissues was observed (Fig. 1). Root infestation, general root tissue or vascular system infection, and root pruning processes were distinguishable and were followed weekly. Plant height measurements and assessment of various plant and pathogen growth characteristics, e.g., leaf and root numbers and condition, severity of infection, formation of various pathogen structures, etc., were made repeatedly on each plantlet during the study period. In addition, some tubes were inoculated with more than one pathogen, which allowed observations of the interactions between them as well as host tissue responses. Permanent records of these various processes were easily obtained with a 35-mm camera mounted on the microscope.

This technique is not proposed to replace other approaches, but rather to complement other techniques, e.g., greenhouse or growth chamber studies, in which destructive sampling is currently the only means of observing pathogen–host interactions in detail. As this is an artificial system, results derived from the tissue culture plantlet studies should be confirmed with other systems. However, it does offer some advantages over other techniques. For example, microorganism interactions and host responses can be repeatedly observed microscopically, as destructive sampling is not required. A hand-held or microscope-mounted video camera also has been successfully used for recording various events.

This technique is easily established and less costly in terms of labor, space, and materials than conventional greenhouse or growth chamber studies. For potatoes and several other plant species, tissue culture laboratories have tissue culture plantlets available, eliminating the necessity to produce them. For plant species with limited aerial growth, small plastic boxes or other containers with one or more plantlets are often available. The technique also may be useful as a preliminary screening method for host resistance in plant breeding–selection programs (3,4,5). Furthermore, since samples of host tissues and pathogen structures can be easily removed under sterile conditions or the whole plantlet removed after gentle warming to melt the agar, various histological, cytological, and chemical assessments and other investigations may be performed. Other applications are no doubt possible, including those involving chemical amendments to the growth medium and/or plant foliage or other modifications for specific studies on unique pathogen–host or multiple pathogen–host systems.

LITERATURE CITED

1. Anderson, A. J., and Guerra, D. 1985. Responses of bean to root colonization with *Pseudomonas putida* in a hydroponic system. Phytopathology 75:992-995.
2. Benhamou, N., and Côté, F. 1992. Ultrastructure and cytochemistry of pectin and cellulose degradation in tobacco roots infected by *Phytophthora parasitica* var. *nicotianae*. Phytopathology 82:468-478.
3. Gray, L. E., Guan, Y. Q., and Widholm, J. 1987. Use of tissue culture for evaluating soybeans for resistance to *Phialophora gregata*. (Abstr.) Phytopathology 77:1746.
4. Hartman, C. L., Albaugh, D. A., Secor, G. A., and Venette, J. R. 1987. In vitro screening of dry bean calli to identify resistance to *Sclerotinia sclerotiorum*. (Abstr.) Phytopathology 77:1773.
5. Hartman, C. L., and Secor, G. A. 1985. Comparative response of potato leaves, tuber tissue and stem callus to culture filtrates of *Verticillium* spp. (Abstr.) Phytopathology 75:1377.
6. Mauk, P. A., and Hine, R. B. 1988. Infection, colonization of *Gossypium hirsutum* and *G. barbadense*, and development of black root rot caused by *Thielaviopsis basicola*. Phytopathology 78:1662-1667.
7. Murashige, T., and Skoog, F. 1962. A revised medium for rapid growth and bio-assays with tobacco tissue cultures. Physiol. Plant. 15:473-497.
8. Randhawa, P. S., and Schaad, N. W. 1985. A seedling bioassay chamber for determining bacterial colonization and antagonism on plant roots. Phytopathology 75:254-259.