THE APPLICATION OF AN EXCISED ROOT ASSAY FOR THE DETERMINATION OF SUSCEPTIBILITY OR RESISTANCE TO ROOT-KNOT NEMATODES (*MELOIDOGYNE* SPP. GOELDI) IN POTATOES (*SOLANUM* SPP. L.)

Catherine E. Rivera-Smith[1], Howard Ferris[2], and Ronald E. Voss[3]

Abstract

Excised roots of six potato selections were challenged with egg inoculum of four *Meloidogyne* species: *M. chitwoodi, M. javanica, M. incognita,* and *M. hapla.* The dual cultures were established under aseptic conditions in 60 × 15 mm petri plates on a defined agar medium. Egg hatch occurred within one week and galling within four weeks at incubation temperature of 24°C. All six potato selections, Russet Burbank, White Rose, PAS 3065, *S. sparsipilum* 365343.A19-12, *S. sparsipilum* × *S. phureja* 856D68-1, and NDTX8731-1R supported reproduction of all four *Meloidogyne* species. Corresponding greenhouse tests were run with each *in vitro* experiment to establish the efficacy of this screening technique compared to greenhouse methods. Results from the greenhouse assays had less variation within cultivar treatment than did the results from the *in vitro* assays. The *in vitro* assay, however, may be conducted with less labor, time, and greenhouse facility management.

Compendio

Se expusieron raíces cortadas de seis selecciones de papa al inóculo (huevos) de cuatro especies de *Meloidogyne*: *M. chitwoodi, M. javanica, M. incognita* y *M. hapla.* Cultivos duales fueron establecidos bajo condiciones asépticas en platos de petri de 60 × 15 mm con un medio definido de agar. La eclosión de los huevos se produjo en una semana y la formación de agallas en cuatro semanas a una temperatura de incubación de 24°C. Las seis selecciones de papa, Russet Burbank, White Rose, PAS 3065, *S. sparsipilum* 365343.A19-12, *S. sparsipilum* × *S. phureja* 856D68-1 y NDTX8731-1R permitieron la reproducción de las cuatro especies de *Meloidogyne.* Las pruebas

[1]Former graduate student, Departments of Nematology and Vegetable Crops, University of California, Davis, CA 95616.
[2]Professor and Nematologist, Department of Nematology, University of California, Davis, CA 95616.
[3]Extension Vegetable Crops Specialist, Department of Vegetable Crops, University of California, Davis, CA 95616.
Present address of first author: Summit Plant Laboratories, Inc., 2301 Research Blvd., Suite 106, Ft. Collins, CO 80526.
Accepted for publication August 24, 1990.
ADDITIONAL KEY WORDS: *M. chitwoodi, M. hapla, M. incognita, M. javanica.*

correspondientes de invernadero fueron llevadas a cabo con cada experimento *in vitro* para establecer la eficacia de esta técnica de evaluación y selección en comparación con los métodos de invernadero. Los resultados de las pruebas en el invernadero variaron menos dentro del tratamiento de cultivares que los resultados obtenidos de las pruebas *in vitro*. Sin embargo, la prueba *in vitro* puede conducirse con menos trabajo, tiempo y manejo de las instalaciones de invernadero.

Introduction

Potato breeding programs commonly involve the observation of thousands of genotypes in greenhouse bioassays in order to identify new selections which exhibit resistance to root-knot nematodes (*Meloidogyne* spp.) (7, 9, 11). Orion and Pilowsky (14) have reported the success of an *in vitro* excised root assay which accurately identified tomato (*Lycopersicon esculentum* Mill) cultivars with known susceptibility and resistance to *Meloidogyne incognita* (Kofoid and White) Chitwood. The utilization of a laboratory assay for the initial screening of potato genotypes for root-knot nematode resistance could represent considerable savings in time, facilities, and labor. The reported effectiveness of applying tissue culture in other potato improvement efforts (5, 19) suggests that the application of an excised root assay for nematode resistance is feasible.

In this study, two types of bioassays were conducted. A greenhouse experiment and an *in vitro* excised root experiment were conducted utilizing six potato selections challenged separately with four species of root-knot nematode. Susceptibility, in terms of the host efficiency of each potato selection, was measured by the ratio (P_f/P_i) of the final count of *Meloidogyne* eggs extracted to the initial count introduced as inoculum (3). In addition, a gall rating (based on percent of the root system distorted by root-knot galls) was assigned to characterize the plant response in both types of bioassays (7). The objective in conducting the parallel bioassays was to compare the results of both types of tests and thereby establish the effectiveness of an excised root assay.

Materials and Methods

Establishment of Excised Root Cultures

Five of the potato selections used in the study were cultured from node or tuber sprout cuttings from single plant or tuber selections (sel.). These included the cvs Russet Burbank, White Rose, *S. sparsipilum* (Britt). Juz. et Buk. selection 365343.A19-12, a cross between *S. sparsipilum* and *S. phureja* Juz. et Buk. (hereafter referred to as 68-1), and *S. tuberosum* selection NDTX8731-1R. Surface disinfestation involved a 1% sodium hypochlorite and 70% ethanol treatment, followed by three sterile rinses (18). Tissues were agitated with an ultrasonic cleaner.

Sterile, *in vitro* seedlings were also established using true potato seed of breeding sel. PAS 3065. Several seeds were soaked in 0.5% sodium hypochlorite solution and rinsed three times with sterile water. A single vigorous seedling was selected for subsequent clonal propagation.

Plant and tuber cuttings and true potato seed plantlets were established on Murashige and Skoog (MS) medium (12). Every 21 days, the rooted shoot cultures were dissected into single node and shoot tip cuttings and subcultured onto fresh MS media plates (ten cuttings per 100 × 15 mm plate). The culture plates were double-wrapped with "parafilm" and incubated at 22°C with 16-hour light.

Root tip sections of approximately 0.5 cm were transferred from established rooted shoot cultures to 60 × 15 mm petri plates with Skoog, Tsui, and White (STW) medium (17). Three tips were transferred per plate. Root plate cultures were grown at 24°C in complete darkness for 10 days prior to inoculation with *Meloidogyne* spp.

Inoculation of Excised Root Cultures with Meloidogyne *spp.*

Confirmation of the root-knot nematode species used in each experiment was based on microscopic examination of perineal patterns as described by Riggs (1985). *Meloidogyne javanica* (Treub) Chitwood, *M. hapla* Chitwood, and *M. incognita* were taken from a species collection maintained on infected greenhouse tomato plants. *M. chitwoodi* Golden, O'Bannon, Santo and Finley, was obtained from infected potato plants from a field in the Klamath Basin region of Northern California.

Approximately 75 to 100 egg masses were removed from infected roots with forceps. The gelatinous egg matrix was dissolved in a 0.5% sodium hypochlorite solution for four minutes as described by Barker (2). After rinsing and collecting eggs on a 500-mesh (0.26 mm aperture) screen, the separated eggs were surface disinfested with a 2-minute soak in 0.001% HgCl (10), followed by three sterile rinses. After making a sample count from a 100μl aliquot, 5.0 to 8.0 ml of sterile water were used to prepare a *Meloidogyne* spp. suspension of known concentration, and 300 eggs were uniformly applied across 12 root plates for each potato selection.

The inoculated root plates were incubated in the dark at 24°C. After 10 weeks, roots were removed from the agar plates, weighed, and vortexed in test tubes with a 1% sodium hypochlorite solution to disrupt the gelatinous matrix and release the eggs. The eggs extracted from each root culture plate were counted to establish the eggs per gram of root produced and the P_f/P_i ratio.

Greenhouse Assays

The same clonal material used in the *in vitro* experiments was transferred out of sterile culture following routine subculture. Tissue culture plantlets were hardened in a plastic box for one week before transplanting to 414 cm^3 styrofoam cups filled with an autoclaved 9:1 sand-clay mixture.

After two weeks growth in the greenhouse, five plants of each potato selection were inoculated with 1000 root-knot nematode eggs per pot. The same inoculum source that was used in each laboratory assay was also used in the corresponding greenhouse experiment. The greenhouse assays utilizing *M. chitwoodi* and *M. incognita* as inoculum were conducted during late summer and early fall with no supplemental light. The assays using *M. hapla* and *M. javanica* were conducted during spring months, and daylength was supplemented with fluorescent lights to total 16 hours daylight. Greenhouse temperatures ran at 25-28°C during the day and 24°C at night.

Greenhouse experiments were evaluated 12 weeks following inoculation. The root system of each plant was rinsed free of soil, blotted dry with paper towels, and weighed. The nematode eggs from each root system were extracted using a 1% sodium hypochlorite solution and single arm flask shaker agitation for 5 minutes. The eggs extracted from each sample were counted to determine the eggs produced per gram of root. The P_f/P_i ratio was recorded as being greater or less than 1, indicating whether or not the nematode population had increased. Root weights and eggs per gram were analyzed in a one-way analysis of variance. Means from both data sets were compared with Duncan's multiple range test.

Results

Establishment of Excised Root Cultures with Nematode Inoculum

In all four tissue culture experiments, egg hatch began 3-4 days following inoculation. The range of egg hatch in each excised root experiment was 20-30%. The maximum number (35-50) of active, second-stage juveniles was observed between the fourth and eighth day after inoculation. Less than 0.5% of the replicates in any one tissue culture experiment were lost due to contamination.

All six potato selections used in these experiments formed root galls in the fourth to sixth week of incubation. The *S. sparsipilum* sel., 365343.A19-12, and sel. 68-1, were consistently the first to show galling. Galls appeared on root plates of Russet Burbank up to two weeks later than any other potato selection. This pattern of galling was consistent in each experiment using each of the four *Meloidogyne* species as inoculum (Fig. 1A-C).

Comparison of Results from the Excised Root Assays and the Greenhouse Assays

In nearly all instances, all six potato selections used in this study appeared highly susceptible to each of the four *Meloidogyne* species when the assays were conducted in the greenhouse. The only exception was the response of Russet Burbank to *M. chitwoodi*, where two of the five replicates died early in the experiment, and only one of the remaining three replicates galled significantly and yielded a P_f/P_i ratio > 1.

When compared to the greenhouse assays, the excised root assays were less consistent in detecting the susceptibility of the potato materials tested.

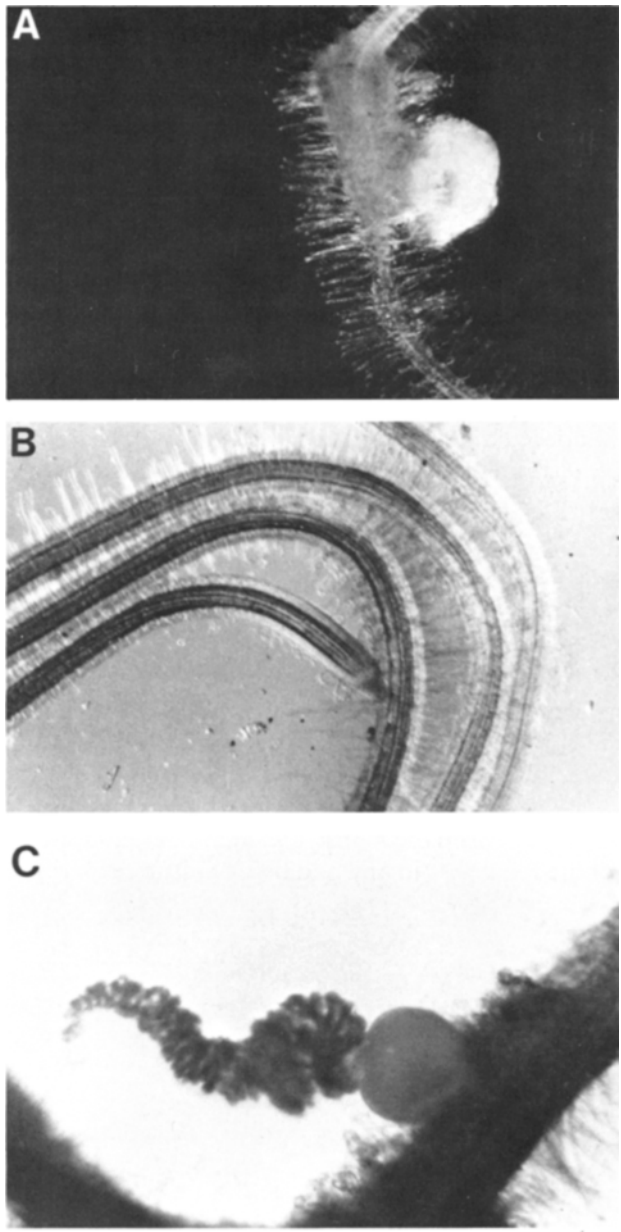

FIG. 1. Galling response on excised roots and the development of female root-knot nematode *in vitro* (magnification 100×).

A) Typical galling response on Russet Burbank roots infected by *Meloidogyne chitwoodi*.
B) Appearance of non-inoculated roots of Russet Burbank *in vitro*.
C) *Meloidogyne chitwoodi* female with attached egg sac external to the excised roots of *Solanum sparsipilum*.

Individual replicates of Russet Burbank, White Rose, PAS 3065, and selection NDTX8731-1R produced galls, egg masses, and a P_f/P_i ratio > 1. However, an equal or greater number of replicates of the same selections in a given experiment produced little or no response. Only the *S. sparsipilum* sels. 365343.A19-12 and 68-1 consistently produced a high number of galls and a P_f/P_i ratio > 1 across replicates comparable to the corresponding greenhouse assay (Tables 1-4). Depending on the cultivar, females on greenhouse roots produced 3 to 90 times the eggs per gram root produced by females sustained on excised roots (Table 5).

Discussion

The susceptibility of the six potato selections used in this study to four root-knot nematode species was determined using greenhouse and excised root assays. The terminology for susceptibility and resistance applies to the host-parasite system, as proposed by Canto-Saenz (3): "resistance" is defined as a non-efficient host that suffers no statistically significant damage, and "susceptible" is defined as an efficient host that suffers statistically significant damage.

It was expected that the commercial cultivars of *S. tuberosum* (Russet Burbank and White Rose, the true potato seed selection of PAS 3065, and the breeding selection NDTX8731-1R) would be susceptible to the *Meloidogyne* species tested. *S. sparsipilum* sel. 365343.A19-12 had been previously scored as resistant in greenhouse screening tests, and was included in the design of these experiments as a resistant control. The cross between *S. sparsipi-*

TABLE 1.—*Comparison of results from excised root assay versus greenhouse assay using* Meloidogyne chitwoodi *as inoculum.*

Potato selection	Excised root assay[a]			Greenhouse assay[b]		
	P_f/P_i>1	*Gall rating	% Replicates scored susceptible	P_f/P_i>1	*Gall rating	% Replicates scored susceptible
68-1	Yes	4	40%	Yes	4	100%
A19-12 *sparsipilum*	Yes	4	75%	Yes	4	100%
PAS 3065	Yes	3	25%	Yes	4	100%
NDTX8731-1R	Yes	3	33%	Yes	4	100%
Russet Burbank	Yes	2	25%	Yes	4	20%
White Rose	Yes	3	67%	Yes	4	100%

[a]12 replicates.
[b]5 replicates.
*4 = severe root enlargement and galling (80-90%).
3 = slight to moderate root enlargement and galling (40-50%).
2 = slight root enlargement, few galls (10-20%).
1 = no root enlargement or galling (<10%).

lum and *S. phureja* was included to test for the transference of resistance, given the conclusions by Gomez, *et al.* (8) that resistance to *M. incognita, M. javanica,* and *M. arenaria* exists in *S. sparsipilum.* Because all of these cultivars and selections became galled and supported reproduction in all assays, this study did not establish that the excised root assay could be used to determine re-

TABLE 2.—*Comparison of results from excised root assay versus greenhouse assay using* Meloidogyne incognita *as inoculum.*

Potato selection	Excised root assay[a]			Greenhouse assay[b]		
	$P_f/P_i>1$	*Gall rating	% Replicates scored susceptible	$P_f/P_i>1$	*Gall rating	% Replicates scored susceptible
68-1	Yes	4	100%	Yes	4	100%
A19-12	Yes	4	100%	Yes	4	100%
sparsipilum						
PAS 3065	Yes	3	42%	Yes	4	100%
NDTX8731-1R	Yes	3	33%	Yes	4	100%
Russet Burbank	Yes	2	17%	Yes	4	100%
White Rose	Yes	3	75%	Yes	4	100%

[a]12 replicates.
[b]5 replicates.
*4 = severe root enlargement and galling (80-90%).
3 = slight to moderate root enlargement and galling (40-50%).
2 = slight root enlargement, few galls (10-20%).
1 = no root enlargement or galling (<10%).

TABLE 3.—*Comparison of results from excised root assay versus greenhouse assay using* Meloidogyne hapla *as inoculum.*

Potato selection	Excised root assay[a]			Greenhouse assay[b]		
	$P_f/P_i>1$	*Gall rating	% Replicates scored susceptible	$P_f/P_i>1$	*Gall rating	% Replicates scored susceptible
68-1	Yes	4	83%	Yes	4	100%
A19-12	Yes	4	75%	Yes	4	100%
sparsipilum						
PAS 3065	Yes	2	17%	Yes	4	80%
NDTX8731-1R	Yes	2	8%	Yes	4	100%
Russet Burbank	Yes	2	8%	Yes	4	100%
White Rose	Yes	4	75%	Yes	4	100%

[a]12 replicates.
[b]5 replicates.
*4 = severe root enlargement and galling (80-90%).
3 = slight to moderate root enlargement and galling (40-50%).
2 = slight root enlargement, few galls (10-20%).
1 = no root enlargement or galling (<10%).

TABLE 4.—*Comparison of results from excised root assay versus greenhouse assay using* Meloidogyne javanica *as inoculum.*

Potato selection	Excised root assay[a]			Greenhouse assay[b]		
	$P_f/P_i>1$	*Gall rating	% Replicates scored susceptible	$P_f/P_i>1$	*Gall rating	% Replicates scored susceptible
68-1	Yes	4	92%	Yes	4	100%
A19-12 *sparsipilum*	Yes	4	100%	Yes	4	100%
PAS 3065	Yes	3	42%	Yes	4	100%
NDTX8731-1R	Yes	4	67%	Yes	4	100%
Russet Burbank	Yes	3	50%	Yes	4	100%
White Rose	Yes	4	83%	Yes	4	100%

[a]12 replicates.
[b]5 replicates.
*4 = severe root enlargement and galling (80-90%).
3 = slight to moderate root enlargement and galling (40-50%).
2 = slight root enlargement, few galls (10-20%).
1 = no root enlargement or galling (<10%).

TABLE 5.—*Comparison of eggs per gram of root from an excised root assay and a greenhouse assay using* Meloidogyne javanica *as inoculum.*

Potato selection	Excised root assay[a]		Greenhouse assay[b]	
	Root weight (grams)	Eggs/gram* root	Root weight (grams)	Eggs/gram* root
68-1	0.15±0.02	11.5±2.7 abc	6.60±0.95	37.0± 5.1 a
A19-12 *sparsipilum*	0.15±0.02	17.1±4.6 a	1.58±0.70	83.6± 5.4 a
PAS 3065	0.03±0.01	0.8±0.4 d	2.60±1.15	76.6±21.5 a
NDTX8731-1R	0.05±0.01	13.3±4.0 ab	3.22±0.61	101.2±20.8 a
Russet Burbank	0.01±0.01	4.3±1.2 cd	3.81±0.73	83.8±21.3 a
White Rose	0.09±0.01	7.2±2.0 bcd	1.23±0.02	78.4±31.4 a

[a]The average of 12 replicates.
[b]The average of 5 replicates.
*Number in thousands of eggs. Values not followed by the same letter in each group were different, P=0.05 (Duncan's multiple range test).

sistance to the root-knot nematode. The excised root assay did detect the susceptibility of each potato selection found to be susceptible in the corresponding greenhouse assay (Tables 1-4). Comparison of the eggs per gram of root, however, revealed that reproduction is not supported on the excised roots at a rate comparable to that on a whole plant root (Table 5). The limitation of the excised root assay, as conducted in this study, appears

to be releated to the adequacy of the excised potato root to be an efficient host for the nematode.

Potato roots excised from rooted shoot cultures will grow on STW medium; however, the results of the four *in vitro* assays, reported here, suggest that *S. tuberosum* selections fail to extract adequate nutrition to support the level of root-knot nematode development found in these same selections in the greenhouse. At the end of the experimental period, ungalled material taken from excised root plates of the *S. tuberosum* cultivars contained numerous undeveloped second-stage juveniles observable by light microscopy after staining. In contrast, excised roots from the wild *Solanum* species consistently showed galling and produced the highest number of eggs per gram root in the excised root experiments. Unlike tomato roots, roots excised from potato plants cannot be continuously cultured as root cultures without the addition of plant growth regulators and/or potato extract, which is reported to contain unknown factors which enhance root growth (1, 4). Orion, *et al.* (13) avoided both the inhibitory effects that high ammonium nitrate has on development of female root-knot nematodes, and the enhancement of plant susceptibility which can result from the application of exogenous plant growth regulators (6, 13, 16).

Some modification of the root culture medium must be made to accommodate the needs of all the *Solanum* species most likely to be included in a breeding program for resistance to root-knot nematode. The addition of a potato extract, as described by Chapman (4), and an increase of the major salt nutrient compounds in the STW medium might improve root response without adversely affecting nematode development.

This study established that four *Meloidogyne* spp. known to infect potatoes can be observed throughout the normal life cycle of the pest on a dual culture system *in vitro*. The advantages of applying such a laboratory bioassay both for the study of host-parasite interaction and for the identification of resistant potato genotypes are numerous. Observations and evaluations can be made using an excised root assay without the interference of insect pests or microorganisms. Observations can be made continuously and in a non-destructive fashion. The control of temperature and water relations is much more exacting under incubator conditions than in a greenhouse. In a single incubator, hundreds of culture plates can be stored without the facilities and continuous labor requirements of a greenhouse assay of equal size. For these reasons, further efforts to develop an excised root assay for the determination of susceptibility or resistance to root-knot nematodes and other root crop pests could prove most beneficial.

Literature Cited

1. Bajaj, Y.P.S. and L.A. Dionne. 1968. The continuous culture of excised potato roots. N Z J Bot 6:386-394.

2. Barker, K.R. 1985. Nematode extraction and bioassays. *In:* Sasser, J.N. and C.C. Carter (Eds.). Advanced Treatise on *Meloidogyne*. Vol. 1. Biology and Control. North Carolina State University, Raleigh, NC. pp. 19-35.
3. Canto-Saenz, M. 1985. The nature of resistance to *Meloidogyne incognita*. *In:* Sasser, J.N. and C.C. Carter (Eds.). Advanced Treatise on *Meloidogyne*. Vol. 1. Biology and Control. North Carolina State University, Raleigh, NC. pp. 225-231.
4. Chapman, H.W. 1956. Continuous growth of excised potato root tips. Am J Bot 43:468-471.
5. Dodds, J.H. 1988. Tissue culture technology: practical application of sophisticated methods. Am Potato J 65:167-179.
6. Dropkin, V.H., J.P. Helgeson and C.D. Upper. 1969. The hypersensitivity reaction of tomatoes resistant to *Meloidogyne incognita*: reversal by cytokinins. J Nematol 1:55-61.
7. Fassuliotis, G. 1979. Plant breeding for root-knot resistance. *In:* Lamberti, F. and C.E. Taylor (Eds.). Root-knot Nematodes (*Meloidogyne* species). Academic Press, London, pp. 425-453.
8. Gomez, P.L., R.L. Plaisted and B.B. Brodie. 1983. Inheritance of the resistance to *Meloidogyne incognita*, *M. javanica*, and *M. arenaria* in potatoes. Am Potato J 60:339-352.
9. Howard, H.W. 1970. Genetics of the potato. Springer-Verlag, New York. pp. 89-97.
10. Huettel, R.N. and R.V. Rebois. 1985. Culturing plant parasitic nematodes using root explants. *In:* Zuckerman, B.M., W.F. Mai, and M.B. Harrison (Eds.). Plant Nematology Laboratory Manual. University of Massachusetts Agricultural Experiment Station, Amherst, MA. pp. 155-158.
11. Jatala, P. 1983. An efficient method for screening large numbers of potato seedlings for resistance to *Meloidogyne* species. *In:* Hooker, W.J. (Ed.). Research for the Potato in the Year 2000. International Potato Center, Lima, Peru. pp. 105-106.
12. Murashige, T. and F. Skoog. 1962. A revised medium for rapid growth and bio assays with tobacco tissue cultures. Physiol Plant 15:473-497.
13. Orion, D., W.P. Wergin and B.Y. Endo. 1980. Inhibition of syncytia formation and root-knot nematode development on culture of excised tomato roots. J Nematol 12:196-203.
14. Orion, D. and M. Pilowsky. 1984. Excised root culture as a tool for testing root knot nematode resistance. Phytopathology 12:71-73.
15. Riggs, R.D. 1985. Making perineal patterns of root knot nematodes and vulval cones of cyst nematodes. *In:* Zuckerman, B.M., W.F. Mai and M.B. Harrison (Eds.). Plant Nematology Laboratory Manual. University of Massachusetts Agricultural Experiment Station, Amherst, MA. pp. 197-199.
16. Sawhney, R. and J.M. Webster. 1975. The role of plant growth hormones in determining the resistance of tomato plants to the root-knot nematode, *Meloidogyne incognita*. Nematologica 21:95-103.
17. S'Jacob, J.J. and J.V. Besooijen. 1971. A manual for practical work in nematology. International post-graduate nematology course. Wageningen. pp. 50-52.
18. Wambugu, F.M., G.A. Secor and N.C. Gudmestad. 1985. Eradication of potato virus Y and S from potato by chemotherapy of cultured axillary bud tips. Am Potato J 62:667-672.
19. Wang, P. and C. Hu. 1985. Potato tissue culture and its applications in agriculture. *In:* Li, P.H. (Ed.). Potato Physiology. Academic Press, Orlando, FL. pp. 503-577.