Potential of Tissue Culture for Breeding Root-knot Nematode Resistance into Vegetables[1]

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Abstract: Plant protoplast technology is being investigated as a means of transferring root-knot nematode resistance factors from *Solanum sisymbriifolium* into the susceptible *S. melongena*. *Solanum sisymbriifolium* plants regenerated from callus lost resistance to *Meloidogyne javanica* but retained resistance to *M. incognita*. Tomato plants cloned from leaf discs of the root-knot nematode resistant 'Patriot' were completely susceptible to *M. incognita*, while sections of stems and leaves rooted in sand in the absence of growth hormones retained resistance. Changes in resistance persisted for three generations. It is postulated that the exogenous hormonal constituents of the culture medium are modifying the expression of genetic resistance. *Key words:* review, protoplast, callus, *Solanum sisymbriifolium*, *Solanum melongena*, eggplant, tomato, *Meloidogyne incognita*, *Meloidogyne javanica*, root-knot nematodes, somatic hybridization.

Journal of Nematology 14(1):10-14. 1982.

It is generally accepted that polygenic horizontal resistance to nematodes would be more stable and effective for longer periods of time than vertical resistance conditioned by a single gene. This, in turn, is dependent upon the availability of sexually compatible and genetically diverse germ plasm sources. The gene pool in some crops for root-knot nematode resistance is large, and resistant plants can be readily identified for use in a plant breeding program. In others, the variability is either extremely narrow or nonexistent, necessitating the search for resistance in related species, which most often are incompatible with the cultivated species (7).

The eggplant (*Solanum melongena*), for example, is a vegetable crop in which no resistance to root-knot nematodes has been found (4). A potential source of resistance occurs in a related wild species, *S. sisymbriifolium*, (10). However, the two species are sexually incompatible. In order to transfer the resistance factors of the wild species into the eggplant, methods other than conventional breeding techniques would have to be utilized.

Recent advances in plant protoplast technology provide a mechanism for overcoming interspecific and intergeneric barriers to hybridization and for introducing new genetic information into plant cells without sexual reproduction. Production of a hybrid plant through protoplast fusion of vegetative cells is commonly referred to as somatic hybridization (2).

Recent reports on the hybridization of the potato (*Solanum tuberosum*) with the tomato (*Lycopersicon esculentum*) and that of *Arabidopsis* and *Brassica* by protoplast fusion have demonstrated the feasibility of this technology for hybridizing sexually noncompatible plant species (12,14).

In somatic hybridization, plant tissues from two parental sources are digested with a mixture of pectinase and cellulase in an osmotically stable solution to produce protoplasts. Protoplasts are naked cells surrounded by a plasm membrane which have the capacity to fuse spontaneously or to be induced to fuse by mixing them together in the presence of a high molecular weight polyethylene glycol (PEG). A small percentage of the protoplasts will fuse to form heterokaryons containing a mixed cytoplasm with two nuclei. The fused protoplasts are capable of cell wall regeneration, growth, and division. After cell division a single nucleus may be formed containing the chromosomes of each parent cell or, more frequently, selective elimination of chromosomes of one of the parental species takes place during cell division and tissue formation (18).

The formation of a true interspecific hybrid cell can be identified readily by fusing green mesophyll protoplasts of one plant partner with the light yellow to colorless protoplasts of another partner prepared from cell suspension cultures. The contrasting colors of the heterokaryons become ob-

Received for publication 1 September 1981.

[1]Symposium presented at the Annual Meeting of the Society of Nematologists, 16-19 August 1981, Seattle Washington. Cooperative investigations, Agricultural Research Service, U. S. Department of Agriculture and South Carolina Agricultural Experiment Station.

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scure after several division and it is next to impossible to identify the hybrid cells from the more numerous parental cells. It is possible, however, to select cell hybrids in a nutrient medium which favors the growth of the hybrid products or preferentially allows the growth of only the hybrid cells (18). The colony of cells is subcultured on a culture medium favoring callus formation. The callus is subsequently transferred to a medium containing an auxin and cytokinin to induce organogenesis.

This technique is being used as a means of transferring root-knot nematode resistance factors from *Solanum sisymbriifolium* into the eggplant, *S. melongena.*

This report presents results of our research leading to the somatic hybridization of the two plant species, but more importantly it presents data on changes in expression of root-knot nematode resistance in cloned plants.

ORGANOGENESIS

Protoplast isolation and culture: Viable clear protoplasts from cell suspension cultures of *S. sisymbriifolum* and green protoplasts from young leaves of eggplant cv. Florida Market were prepared by enzymatic digestion. By manipulating the culture medium with various auxin/cytokinin ratios, osmoticum, light, and temperature, protoplasts from each plant species were induced to form new cell walls, divide repeatedly, produce colonies of cells, and regenerate into whole plants (1).

Heterokaryons: Clear *S. sisymbriifolium* protoplasts were fused with green mesophyll eggplant protoplasts with PEG. The heterokaryons, however, did not divide beyond a few divisions and eventually died. The conditions necessary for sustaining viability and cell divisions of the hybrid cells are being investigated.

Regeneration of plant species from callus: In the early stages of this research callus cultures were established from stem pith parenchyma of *S. sisymbriifolium* and *S. melongena* on Linsmaier and Skoog basal salt medium supplemented with sucrose, indole-3-acetic acid (IAA), (γ,γ-dimethyl-allyl-amino)–purine (2iP) and 2,4-dichlorophenoxyacetic acid (2,4-D). Plants were regenerated from *S. sisymbriifolium* callus on a basal salt medium containing a combination of high concentrations (74.9 μM) of the cytokinin, 2iP, and low concentrations (17.1 μM) of the auxin, IAA (5). Regeneration of *S. melongena* callus required a more complex protocol (11).

Characteristics of plants regenerated from callus: Morphological and cytological aspects of plants regenerated from callus of both *Solanum* species differed from original seed plants. Leaves of regenerated *S. sisymbriifolium* and *S. melongena* were thicker and less deeply lobed and the stomates were larger and contained more chloroplasts than seed plants. Cytological examination of pollen microspores of both species showed 24 chromosomes (n = 12), indicating that they were tetraploids (6,11).

REGENERATED PLANT RESPONSE

Since the plant morphology and cytology varied after regeneration, the regenerated plants were tested for resistance to root-knot nematodes after being selfed for two generations.

The usual response of eggplant roots to infection by *M. incognita* or *M. javanica* is the characteristic galling. The vascular cylinder is disrupted by the development of 6–8 large multinucleate, thick-walled giant cells which surround the nematode's head. Females assume a saccate shape in about 25 days and oviposit a few days later.

Solanum sisymbriifolium is also readily invaded by root-knot larvae. However, instead of galls, roots form swellings, primarily by a hypertrophy of the cortical parenchyma cells. At the nematode feeding site, tracheid continuity is disrupted by the development of small, thin-walled giant cells. Most larvae did not develop beyond the late second stage. The giant cells are not adequate to provide sufficient nutrition for nematode development.

Regenerated plants of both species exhibited the typical phenotypic root response described above after penetration by either *M. incognita* or *M. javanica* larvae. Progeny (S_1 and S_2) of regenerated *S. sisymbriifolium* plants retained resistance to *M. incognita.* Although root swellings were observed, no eggs were recovered. However, regenerated plants were more susceptible than seed plants to *M. javanica.* Larvae developed

into adults at the same rate as in eggplant roots and produced 20 times more eggs than infected *S. sisymbriifolium* seed plants (Table 1). Histological examinations indicated larger giant cells formed in regenerated plant roots than in seed plant roots.

Since the reaction of *S. sisymbriifolium* plants to *M. javanica* was altered, we examined the effect of similar tissue culture techniques on a tomato cultivar containing the *Mi* gene for root-knot nematode resistance. Leaf discs (1-cm diam) of resistant 'Patriot' and susceptible 'Homestead' tomato were cultured on a Murashige and Skoog medium (16) containing 0.1 μM napthaleneacetic acid (NAA) and 10 μM benzyladenine (BA). Plants regenerated from these discs were grown to maturity and S_2 regenerated plants were evaluated for resistance to *M. incognita* after inoculation with 2,000 eggs in 10-cm clay pots (8).

Progeny of plants regenerated from resistant Patriot tomato leaf discs lost their resistance to *M. incognita* (Table 2). The gall and the reproduction indices of the regenerant Patriot did not differ from the reaction of susceptible Homestead tomato plants. Fassuliotis and Deakin (9) observed that hypocotyls of root-knot nematode resistant snap beans supported the development of *M. incognita,* whereas the roots remained resistant. The question, therefore, arose as to whether resistance to root-knot nematodes in the Patriot tomato was expressed in the tops as well as in the roots of the plant, or whether the observed change in resistance was a consequence of the tissue culture procedure.

To answer this question, two experiments were initiated. Stem and leaf cuttings from seed plants of Patriot and Homestead tomato were rooted in sand without applying rooting hormones to the cut surfaces of the tissues. After roots had developed, each cutting was inoculated with 200 *M. incognita* eggs and indexed for galling and reproduction 50 days later (8).

Table 2. Effect of tissue culture of 'Patriot' tomato on resistance to *Meloidogyne incognita* race 1.

Entry	Source	Gall index*	Reprod. index*	Egg/gm root
Patriot	Seed	1.0	1.0	48
Patriot	R.P.†	5.0	4.0	3,715
Homestead	Seed	5.0	5.0	11,128

*Gall index and reproduction index based on a scale of 1–5: 1 = no galling and/or reproduction, 5 = abundant galling and/or reproduction.

†R.P. = S_2 progeny of plants regenerated from leaf discs.

Rooted cuttings from both stem and leaf sections of Patriot tomato retained their resistance to the nematode (Table 3).

In the other experiment, leaf discs from Homestead and Patriot tomatoes were cultured in White's medium containing no phytohormones. Twenty-five *M. incognita* larvae were placed in a drop of water in the center of each disc and incubated at 25 C in the dark for 50 days.

No roots developed from the discs in this medium, but egg-producing females and second-stage larvae were recovered from Homestead tomato leaf discs after 90 days incubation. No females, eggs, or larvae were recovered from Patriot leaf discs.

Table 1. Response of eggplant (*Solanum sisymbriifolium* and *S. melongena*), propagated from seed and tissue culture, to *Meloidogyne incognita* and *M. javanica*.

Host	Source	*M. incognita**			*M. javanica**		
		Gall index	Repr. index	Eggs/gm root	Gall index	Repr. index	Eggs/gm root
S. sisymbriifolium	Seed	2.7	1.0	0.0	3.0	1.0	484
	R.P.†	2.7	1.0	0.0	3.0	4.5	8,715
S. melongena	Seed	4.0	5.0	112,800	4.5	5.0	.....
	R.P.†	4.0	5.0	44,400	4.0	4.5	.....

*Gall index and reproduction index based on a scale of 1–5: 1 = no galling and/or reproduction, 5 = abundant galling and/or reproduction.

†R.P. = regenerated plants, S_2 progeny.

Table 3. The response of rooted cuttings of 'Patriot' tomato to *Meloidogyne incognita* race 1.

Entry	Source	Gall index*	Reprod. index*	Egg/gm root
Patriot	Seed	1.0	1.0	48
Patriot	Stem cuttings	1.0	1.0	0.0
Patriot	Leaf cuttings	1.0	1.0	0.0
Homestead	Seed	5.0	5.0	11,128

*Gall index and reproduction index based on a scale of 1–5: 1 = no galling and/or reproduction, 5 = abundant galling and/or reproduction.

DISCUSSION AND CONCLUSIONS

Despite current optimism for the potential of tissue culture systems for crop improvement, the increase in susceptability or complete loss of root-knot nematode resistance of plants regenerated from callus or tissues of resistant plants suggests that the system has to be approached with extreme caution.

By definition, clonal propagation signifies that all plants so derived from a single mother plant are genetically identical. However, it is not uncommon to obtain aberrant plants after cloning (15). Regeneration of *S. sisymbriifolium,* Florida Market eggplant, and Patriot tomato resulted in plants that differed in their chromosome number, morphology, cytology, and/or expression for root-knot nematode resistance. All of these aberrations have persisted for three generations indicating the induced changes were genetic and not epigenetic events.

Auxins and cytokinins are fundamental ingredients of culture media used for regenerating plants from tissue explants. The formation of roots and shoots is regulated by the availability and appropriate balance of these hormones (15). These materials have also been found to have a profound effect on modifying the expression of root-knot nematode resistance in tomato and peaches (3,13,17).

Within the current state of our knowledge, changes in nematode resistance of *S. sisymbriifolium* and Patriot tomato plants regenerated from tissue culture appear to be induced by the phytohormone constituents of the culture media. But other factors may also be involved. If phytohormones in the media are responsible, then attention must be given to eliminate these effects before tissue culture can be used routinely as a technique for crop plant improvement, as far as root-knot nematode resistance is concerned.

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