NEMATODE ROOT GALLS IN EMBRYO CULTURES OF *CALAMUS ROTANG*

D. Padmanabhan & R. Ilangovan

Department of Plant Morphology, School of Biological Sciences, Madurai Kamaraj University, Madurai - 625 021, India

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PADMANABHAN, D. & ILANGOVAN, R. 1995. Nematode root galls in embryo cultures of *Calamus rotang*. Root galls induced by nematodes were observed in seedlings raised from excised embryos of *Calamus rotang*. Nematode cysts were seen in the cortical cells and in the xylem elements. There was extensive proliferation of outer cortex in the galled part of the root. The aerenchyma between the outer and inner cortex was much reduced. The inner cortex close to the stele had more number of cell layers. The stele showed enlargement. Some cells of the inner cortex developed curious ingrowths, another effect of nematode infection.

Key words: *Calamus rotang* - nematode galls

PADMANABHAN, D. & ILANGOVAN, R. 1995. Puru akar nematod dalam kultur embrio *Calamus rotang*. Puru akar yang disebabkan oleh nematod diperhatikan dalam anak benih yang tumbuh dari embrio *Calamus rotang* yang dipotong. Sista nematod kelihatan di dalam sel-sel kortikal dan di dalam elemen-elemen xilem. Terdapat pembiakan yang banyak di bahagian luar korteks di bahagian puru akar. Bahagian dalam korteks yang dekat dengan stel mempunyai lebih lapisan sel. Stel kelihatan membesar. Sebahagian dari sel di bahagian dalam korteks tumbuh ke dalam. Perkara yang aneh ini merupakan satu lagi kesan infeksi nematod.

Introduction

Nematodes are ubiquitous microscopic animal parasites known to infect roots, causing extensive damage to crops. Some species are known to induce the formation of root galls (Mani 1973). Nematodes could exist and reproduce in callus cultures of crop plants on artificial medium (Schroeder & Jenkins 1963). Recently, the writers encountered nematode root galls in seedlings of *Calamus rotang* grown from excised embryos in sterile culture. Anatomical studies of the gall indicated the presence of nematode cysts confirming their role in gall induction (Christie 1936). The structural changes in the galled portion of the root are reported in this paper.

Material and methods

Mature green fruits of *Calamus rotang* (Palmae) were collected from specimens growing in agriculture fields (known for nematode populations) near the town of Mayiladuthurai in the State of Tamil Nadu, India. The fruits were surface sterilized with detergent solution followed by thorough washing in sterile distilled water. The washed fruits were cut open under aseptic conditions and the embryo isolated and

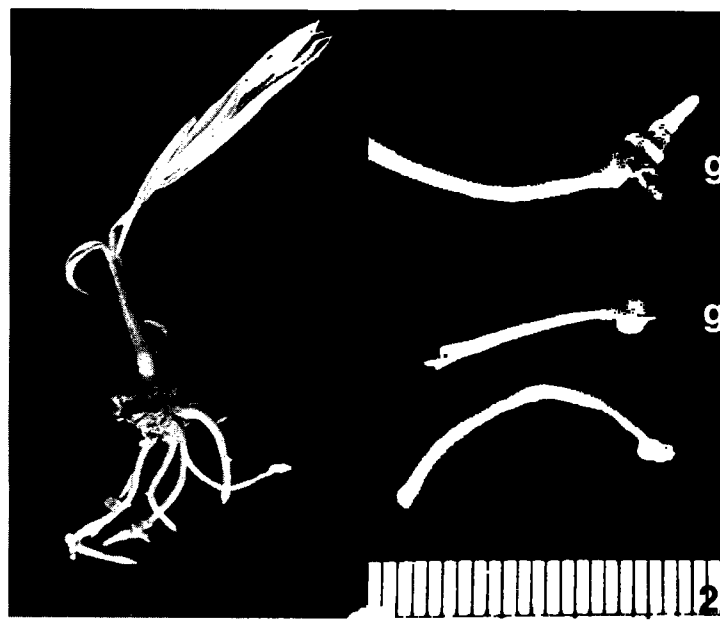
planted on Schenk and Hildebrandt (1972) basal medium supplemented with 3 mg l^{-1} NAA.

As the embryos germinated and formed seedlings *in vitro*, numerous adventive roots appeared. After fifteen days many roots developed galls (swellings) at their tips. The galled roots were fixed in FAA, dehydrated in tertiary butyl alcohol series (Johansen 1940), and embedded in paraffin wax. Serial paraffin sections were cut with a rotary microtome at thicknesses of 8-12 µm. Deparaffinised sections were stained with hematoxylin-safranin combination and mounted in DPX mountant.

Observations and discussion

Growth of the embryo and gall developement

A detailed account of embryo culture in *C. rotang* has already been published (Padmanabhan & Ilangovan 1989). Mature embryos planted on SH basal medium swelled and turned green in 15 - 20 days and the shoot appeared in 30 - 40 days. The haustorium enlarged a little, hardened and turned brown in 30 days. The primary root started growing on the twentieth day and adventive roots appeared 10 days later. After 50 days the adventive roots were about 20 mm long. Galls appeared as swellings near the root tips (Figure 1). Further growth of the roots slowed down as galls were initiated. At this stage (on the 60th day in culture) the material was fixed in FAA.



Calamus rotang. Seedling and nematode galls developed *in vitro*.

Figure 1. The entire seedling removed from the culture tube. Note adventive roots showing swellings - the root galls (x1).

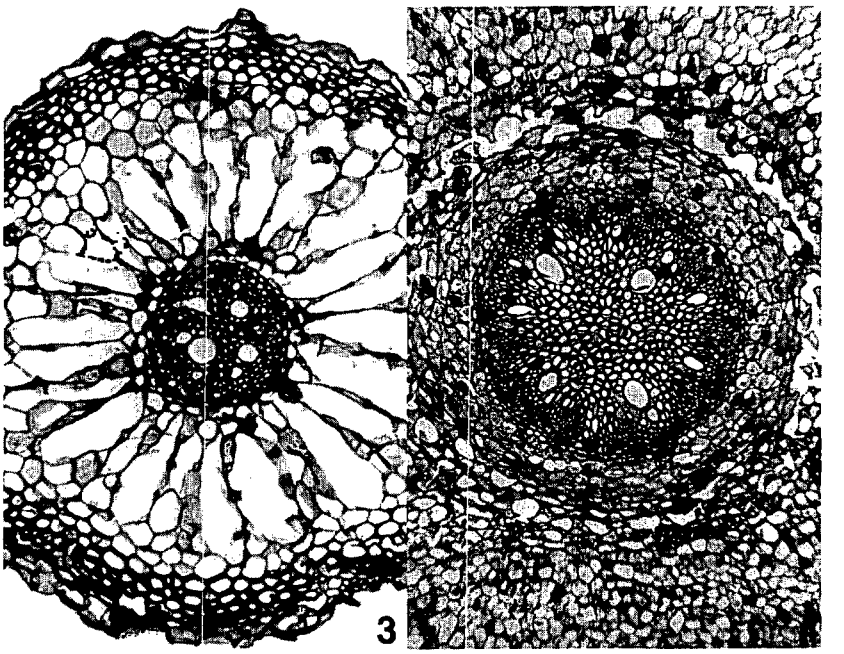
Figure 2. Isolated roots from the seedling (x2). Note the variations in the shape of of galls formed near the root tips (g = gall)

Morphology of the galls

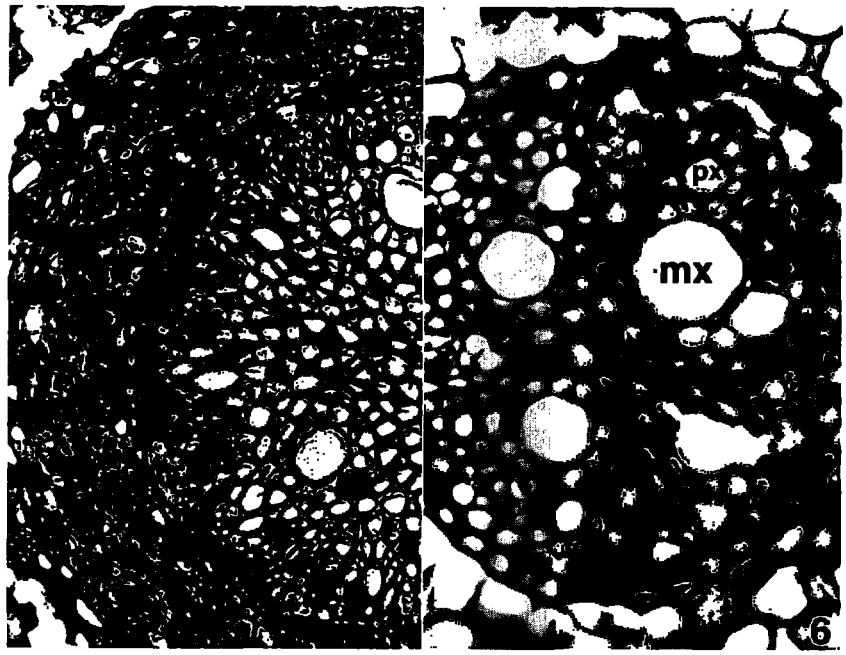
The brownish, ellipsoidal root galls were solid and measured 3 mm in diameter. The roots bearing the galls were 1 mm in thickness (Figure 2).

Anatomy of the roots

The cortex of the primary as well as adventive roots was differentiated into three zones - the outer cortex, the middle aerenchyma and the inner cortex (Figures 3 & 6). A layer of broad epidermal cells covered the roots. The outer cortex was composed of 5-6 layers of small, thick walled sclerenchymatous cells and 3 layers of thin walled cells (Figure 7). The aerenchyma had radiating columns of thin walled parenchyma cells extending between the inner and outer cortex. The inner cortex was composed of about 5 layers of thin walled cells adjoining the endodermis. The central stele measured 137 cm in diameter. The endodermis was a prominent layer composed of drum-shaped cells with thickened walls. The pericycle had 2 - 3 layers of thin walled cells. About 10 groups of protoxylem elements alternated with a similar number of phloem plates in the stele. The number of large metaxylem elements varied from 8 - 10. The central part of the stele was composed of compactly set, small angular cells with highly lignified walls.



Calamus rotang. The anatomy of normal and galled portions of roots.
Figure 3. Transverse section of primary root (normal) showing a compact stele, a broad a aerenchymatous cortex and hypodermal sclerenchyma (x200).
Figure 4. Transverse section of galled portion of root. The black spots near the the protoxylem are cysts formed by nematodes (x63)



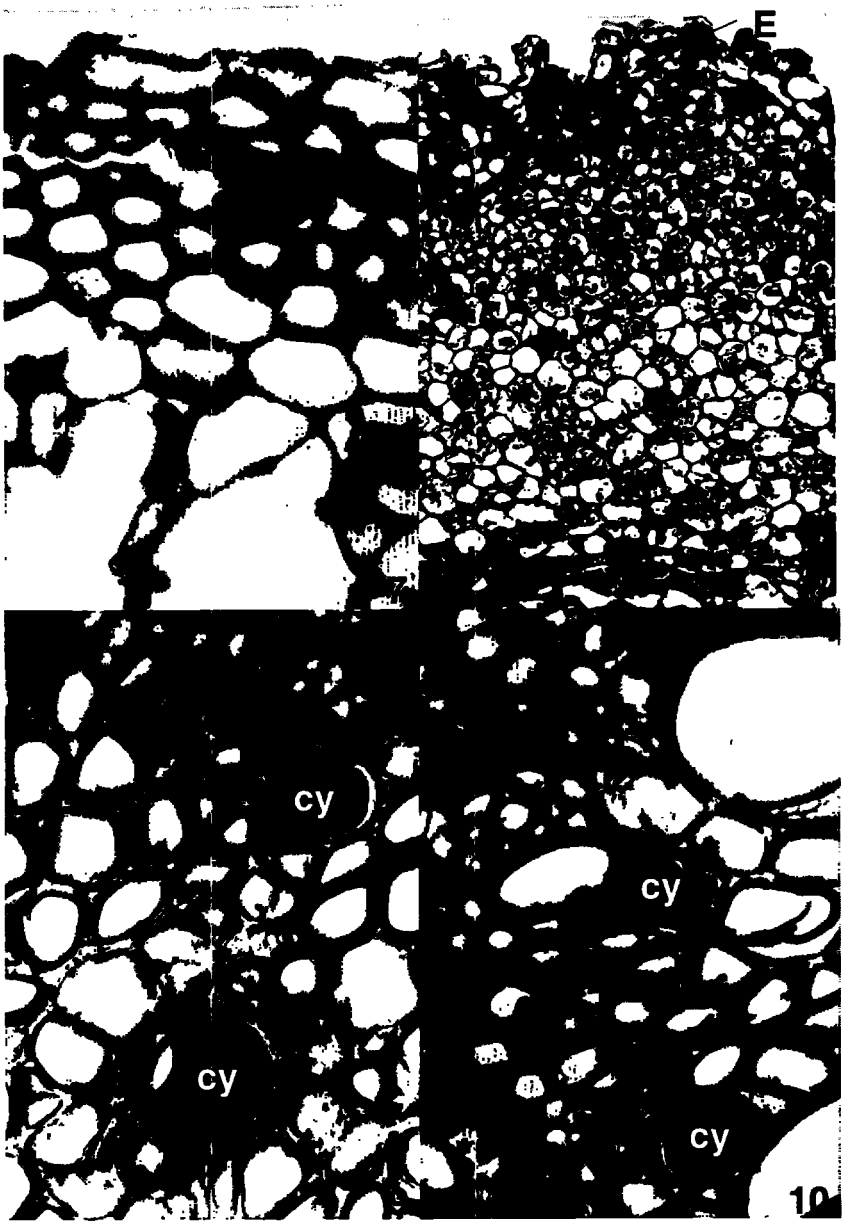
Calamus rotang: The anatomy of normal and galled portions of roots.
Figure 5. An enlarged view of the inner cortex. Note the large intercellular spaces and dense cell contents (x160).
Figure 6. An enlarged view of stele (x533); nematode cysts are absent in the xylem tissue (mx = metaxylem, px = protoxylem)

Anatomy of the galled root

Extensive proliferation of the parenchymatous cells in the inner and outer cortex was a characteristic of the root gall (Figures 4 & 5). The epidermis was ruptured in several places. The subdermal sclerenchyma layer was absent, the concerned cells remaining thin walled and unlignified. About 25 layers of thin walled parenchyma cells could be counted in the outer zone of the cortex. Even though the subdermal cells did not have thick walls as in normal root, the small cells formed a distinct outer zone. Cells of the outer zone harboured bacteria and dark cell contents (Figure 8). The cells of the inner zone were light staining, with large vacuoles (Figures 11-14). Cells with dark tanniniferous contents were seen scattered in the inner zone.

The aerenchymatous middle zone was much compressed; the width being only about 160 cm. The cells spanning the air spaces were not enlarged nor widened. Radiating columns were absent.

The inner cortex was composed of about six layers of compactly arranged parenchymatous cells with dense contents. Conspicuously large intercellular spaces were scattered in this otherwise compact layer. Occasionally, some of these cells had dark tanniniferous cell contents.



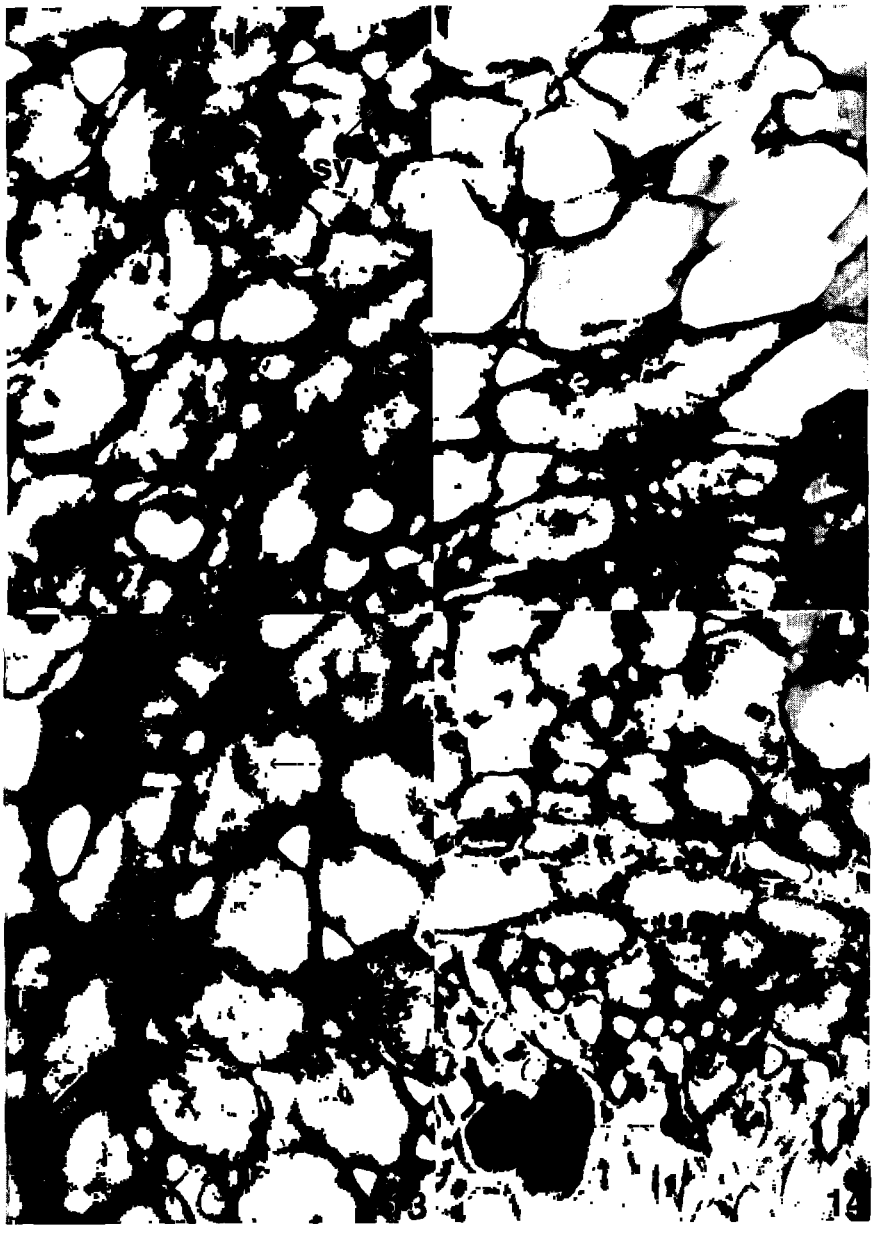
Calamus rotang: Enlarged views of transverse sections through galled roots.

Figure 7. Outer cortex of ungalled part of the root showing sclerenchyma fibres (x 437).

Figure 8. Outer cortex of the galled portion showing proliferation of cortical cells. The cells are aligned in an outer zone of small cells and inner zone of compact cells. Note the granular contents of outer cortex (x 84).

Figure 9. Enlarged view of xylem tissue in a transverse section of the galled portion. Note the prominent nematode cysts within the xylem elemnts (x 327).

Figure 10. Enlarged view of the pericycle zone showing nematode cysts in the protoxylem (x 300) (cy = cyst, E = epidermis)



Calamus rotang. Portions of nematode induced root gall.

Figure 11. Highly magnified view of the inner cortical zone. Note syncytium (arrow) formed due to nematode action. The cells around the endodermis develop peculiar wall projections. Note a cyst in the protoxylem at right bottom (x 610).

Figure 12. Another enlarged portion of cortex showing cells with dense cytoplasm and small vacuoles (x 610).

Figure 13. Another part of cortex enlarged showing bacteria in some cells (arrow) (x 610).

Figure 14. Enlarged view of inner cortex showing endodermis, pericycle and xylem. Note 2 xylem elements with cysts (x 610). (cy = cyst, syn = synctium)

Stele

The stele was much more enlarged in the galled portion compared to the healthy part. The medullary part of the stele was composed of thick walled lignified cells. The vascular system comprised 10 exarch xylem plates alternating with a similar number of phloem strands.

The nematode

Brown cysts of nematodes were found within the thick-walled cells of the xylem (Figures 9 & 10). Some of the cysts in the inner cortex had dark granular contents. Nematode infections are known to induce syncytia in the root. In the material studied some cortical cells were multinucleate (syncytia) (Figure 11). The pericycle cells in the gall developed curious wall ingrowths (Figure 14). Such ingrowths have been noticed in naturally forming galls under field conditions. It is interesting to note that seeds and embryos of wild palms such as *Calamus* could harbour nematodes. Seed borne nematodes show up in root galls in culture. Curious swellings called 'glomerules' were reported in cultures of cotyledenary tubes of the seedlings of *Phoenix dactylifera* (Ammar *et al.* 1985). Sudhersan (1988) recorded similar swellings in embryo cultures of *P. loureii.* The anatomy and etiology of these swellings need further critical studies. It is probable that many wild and cultivated palms carry nematode parasites which could be seed borne. This would be of concern to palm growers and planters.

Acknowledgements

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