Histochemical study of Capsicum annuum L. root galls incited by Meloidogyne incognita Chitwood.

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Abstract. The present investigation deals with the localisation of polysaccharides, RNA, DNA, basic protein and ascorbic acid in the healthy and Meloidogyne incognita infected Capsicum annuum root galls. In general, infected root galls were richer in RNA, DNA, basic protein and ascorbic acid as compared to healthy roots. The nema bodies were also overstained. Thick-walled cells of the infected root are darkly stained. Starch grains were confined to cortical cells; the giant cells and the cells around them were without any starch grains.

Keywords. Meloidogyne incognita; Capsicum annuum; histochemistry.

1. Introduction

Root-knot nematode, Meloidogyne incognita causes heavy damage to various vegetable crops. There are a few biochemical reports on the host–parasitic relationship, of some of the plant parasitic nematodes. Some workers observed that cellulose, free sugars, protein and nucleic acids increased in some crop plants in the galled tissue incited by Meloidogyne spp. (Owens and Novotny 1960; Bird 1961, 1972; Rubinstein and Owens 1964; Littrell 1966; Kannan 1968). Schuster et al (1964) noted the formation of starch grains in the syncytial cells of several plant species induced by Nacobus batatiformis.

2. Materials and methods

Healthy and M. incognita infected root galls of chilli, collected from experimental pots, 45 days after inoculation, were fixed in different fixatives (table 1), dehydrated by tertiary butyl alcohol (TBA) method and embedded in paraffin wax. The serial microtome sections, cut at 12μ, were mounted on slides using Haupt’s adhesive (Jensen 1962). The standard histochemical methods, employed in the localisation of the various substances in the root sections are listed in table 1.
Table 1. Histochemical techniques employed.

<table>
<thead>
<tr>
<th>Metabolite localised</th>
<th>Fixative</th>
<th>Test</th>
<th>Duration of treatment</th>
<th>Colour indication</th>
<th>Control</th>
</tr>
</thead>
<tbody>
<tr>
<td>Total carbohydrate of insoluble polysaccharides</td>
<td>FAA</td>
<td>Periodic acid Schiff's (PAS) method (Jensen 1962)</td>
<td>Periodic acid (0·5%) for 15-25 min at 30°-35°C.</td>
<td>Purplish</td>
<td>By omitting periodic acid step</td>
</tr>
<tr>
<td>Starch</td>
<td>IKI reaction</td>
<td>IKI solution for 5-10 min at 30°-35°C</td>
<td>Blue Black</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Basic protein</td>
<td>10% neutral formalin</td>
<td>Alkaline fast green FCF technique</td>
<td>15% trichloro acetic acid (TCA) at 90°C for 10-15 min</td>
<td>Green</td>
<td>Erection of histones by 0·2 N HCl</td>
</tr>
<tr>
<td>DNA</td>
<td>Carnoy's fluid</td>
<td>Feulgen method</td>
<td>Hydrolysis in 1N HCl at 60°C for 20 min.</td>
<td>Magenta</td>
<td>By omitting hydrolysis</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Schiff's reagent at 30°-35°C for 2 hours</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>RNA</td>
<td>Carnoy's fluid</td>
<td>Pyronin method</td>
<td>Pyronin reagent for 7 min at 30°-35°C</td>
<td>Red</td>
<td>By extracting RNA with 1N perchloric acid for 12-24 hours at 4°C</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Three changes of n-butanol for 5 min each</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Ascorbic acid</td>
<td>Silver nitrate</td>
<td>Silver nitrate method</td>
<td>One week at the low temperature of 0-3°C</td>
<td>Black grains</td>
<td>By oxidation with 5% CuSO₄ solution for 24 hours</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Washed repeatedly in ammonical alcohol</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
3. Observations

3.1. Carbohydrate

(i) Healthy root: Polysaccharides were stained intensely purplish red. However, the cytoplasm remained colourless but the nuclei were feebly stained. The cell walls were not stained in any special manner. Starch grains were abundant in all cells.

(ii) Infected root: In infected roots, polysaccharides were stained intensely purplish red. The cytoplasm was unstained and the nuclei were feebly stained. Thick cell walls of hyperplastic and hypertrophic cells had taken dark stain. Dark stained protuberances occurred in giant cells (figure: 1, 2).

Starch grains were confined to cortical cells. In giant cells and the cells around them, the starch grains were absent (figures 2, 3).

Control: Starch grains were unstained (figure 4).

3.2. DNA

(i) Healthy root: Nuclei of living cells of normal root were stained magenta coloured. The nuclear staining was feeble in normal roots as compared to infected root (figure 8).

(ii) Infected root: Larger giant cell nuclei had many times more DNA than normal cortical cell nuclei. Large irregular nuclei of giant cells and the nematodes were stained magenta coloured (figures 5, 6).

Control: Nuclei remained unstained (figure 7).

3.3. RNA

(i) Healthy root: Cytoplasm of the cells and nucleoli of the nuclei were stained red showing presence of RNA in all the cells.

(ii) Infected root: RNA as detected by pyronin test was concentrated in mature giant cells and in the bodies of the nematodes. As compared to healthy root, the cytoplasm of the infected root cells and the nematodes stained dark red. Nucleoli of giant cell nuclei were stained red (figures 9, 10).

Control: RNA containing regions were unstained (figure 11).

3.4. Basic protein

(i) Healthy root: All the nuclei of healthy root were stained green representing basic proteins (figure 12).

(ii) Infected root: The green stain indicating basic protein was observed in all nuclei of the root tissues. However, prominent irregular nuclei of the giant cells were stained darker green as compared to other cell nuclei. The stain was also concentrated in the nemat bodies (figures 13, 14).

Control: The sections in the control slides were unstained.
3.5. **Ascorbic acid**

(i) **Healthy root**: Infected root cells contained more of ascorbic acid (AA) than normal root cells. Black-brown coloured granules indicating occurrence of AA were noted in all the cells (figure 16).

(ii) **Infected root**: Abundant black-brown and tan black coloured granules were accumulated in infected roots. However, most of the granules occur in giant cells and a few in cortical cell (figure 15).

**Control**: The sections were unstained.

4. **Discussion**

Thick cell walls of hyperplastic and hypertrophic cells were stained intensely purplish red. Thickening may be due to deposition of cellulose (Dropkin and Nelson 1960; Bird 1961; Owens and Novotny 1960; Littrell 1966). Littrell (1966) observed 3 to 23 μ thickened walls of the giant cells incited by *Meloidogyne incognita* in *Hibiscus esculentus*. The thick-walled giant cells bearing prominent protuberances were darkly stained. The nema bodies were also darkly stained.

Starch grains were noted in cortical cells but the giant cells and the cells around them were without starch grains. Absence of starch in the giant cells and the cells around them may be due to secretion of amylase by the nematode that leads to hydrolysis of starch. It seems that the soluble saccharides produced by starch hydrolysis are absorbed by the giant cells. They are probably further broken down to monosaccharides, or to still smaller molecules. These compounds, which are used by the giant cells during their development and highly active metabolism, are ultimately drawn by the nematodes in their process of feeding. Absence of starch in giant cells was also reported by Orion and Bronner (1973), Cohn (1965), Bird (1960) and Owens and Novotny (1960). However, Schuster *et al* (1964) reported starch grains in the giant cells incited by *Nacobus bataiformis* and Sivakumar and Seshadri (1972) in castor cells at the feeding site of *Rotylenchulus reniformis*.

Nuclear material of giant cells and the nematode body is abundant in DNA, whereas RNA was localised in nucleoli of giant cell nuclei, cytoplasm and in the nema bodies. Greater amount of DNA and RNA inside the giant cells may be due to their greater metabolic activity. Owens and Novotny (1960) noted a two-fold increase of nucleic acid in galled tissues and its accumulation both in the giant cells and nema bodies. Bird (1961) and Littrell (1966) observed RNA accumulation in giant cells and in nema bodies as compared to adjacent tissues. Rubinstein and Owens (1964) found 2-11 times more DNA in syncytial nuclei than in cortical cell nuclei.

Basic protein was observed in all the host nuclei but it was more abundant in the irregularly shaped nuclei of the giant cells. The nematode also stained positively for the basic protein. Increase in protein may be due to increased protein synthesis in the giant cells and nema bodies as reported by many workers (Owens and Novotny 1960; Bird 1961, 1972; Littrell 1966; Rubinstein and Owens, 1964; Chylinska and Knypl 1972).

(g, giant cell; fn, female nematode; W, wall; Nu, nucleus; Co, cortex; S, starch grains; X, xylem).
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The present studies agree with Bird (1961), who suggested resemblance between giant cells and meristematic root tip cells in their structure and also in their metabolic activity such as protein synthesis.

More ascorbic acid was noted in galled roots, particularly in giant cells. This shows higher metabolic activity in infected roots. Similar results were obtained by Kannan (1968) in tomato root-knots.

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