

Short note

Biogenic elicitor (arachidonic acid) induced resistance in tomato to *Meloidogyne incognita*

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Biogenic elicitor induced plant resistance to plant parasitic nematodes has been relatively little studied with few data available on plant immunization against phytonematodes. The few published reports refer only to a decrease in the deleterious effects caused by nematodes after plants have been previously infested with less pathogenic species (Nagahama, 1987; Decker et al., 1988). Development of plant protection measures based on the initiation and increase of natural plant defence systems against nematodes offers an environmentally benign disease control strategy.

Recently, the specific role of biogenic elicitors in increasing plant immunological responses to fungi and bacteria has been reported (Kuč, 1995). Elicitors of plant reactions against *Phytophthora infestans*, especially arachidonic acid (AA), have been the most frequently investigated. Concentrations of 0.01–0.1 ppm of AA induced a systemic long-term protection in potato tubers based on the expression of numerous defense genes (Chalova et al., 1989). The resistance mechanism induced by AA in potato involved an adverse action of the final products of modified terpenoid biogenesis on the pathogen in which phytoalexins (PA) toxic to *P. infestans* were produced, rather than phytosterols (PS) required for pathogen metabolism (Kaneva et al., 1993). Rishitin was shown to be responsible for the toxic effect on *P. infestans* both with potato and tomato.

Nematodes are auxotrophic with respect to sterols (Bolla, 1979; Chitwood et al., 1987), as is *P. infestans*, and rishitin is also nematotoxic (Zinovieva & Chalova, 1986). There are several similarities between 'plant-fungus' and 'plant-nematode' interactions, therefore, we considered that AA could be expected to switch the terpenoid metabolism in tomato to make it unfavourable to *M. incognita*. Thus, a natural resistance against this nematode species would be induced. To test our hypothesis we examined the possibility of AA induction of resistance in tomato against *M. incognita*. The induction mechanism was examined by studying the PA and PS contents in treated and control plants. Also, we examined several morphological and physiological characteristics of nematodes to determine if these were affected in immunized plants. The results of our investigation are reported here.

Tomato seeds of cv. F. Karlson, which is susceptible to *M. incognita*, were immersed in AA concentrations of 0.01, 0.1, 1.0, 10.0 and 100 ppm for two hours. After this treatment the seeds were planted individually in 10 cm dia. pots containing sterile soil and were subsequently inoculated with 3000 2nd stage *M. incognita* juveniles per pot (Zinovieva, 1993). Twenty replicates were established for each AA concentration and for the non-treated control. Five days after nematode inoculation into the pots ten plants of each AA concentration were washed free of

Table 1. Influence of arachidonic acid on the control of *Meloidogyne incognita* and immunochemical indices of tomatoes.

| AA concentration ppm | Root-gall rating* | Eggs/g roots (x 100) | Fresh weight (g) | | Content (mg/g) | |
|-------------------------|----------------------|-------------------------|------------------|---------|----------------|--------------|
| | | | Tops | Roots | Sterols | Rishitin |
| 100 | 2.8 bc** | 150.04 b | 37.5 c | 2.33 c | not observed | not observed |
| 10 | 2.6 bc | 147.97 b | 45.5 c | 3.34 c | not observed | not observed |
| 1 | 2.3 c | 25.04 c | 61.7 b | 3.75 b | 0.086 b | 45 c |
| 0.1 | 2.0 c | 30.89 c | 61.2 b | 3.92 ab | 0.090 b | 40 c |
| 0.01 | 3.0 b | 189.23 b | 54.4 a | 4.01 a | 0.097 b | 10 b |
| Control | 4.0 a | 262.67 a | 52.0 a | 4.27 a | 0.175 a | 0 a |

* - Scale: 0 - no galls, 1 - 1-10%, 2 - 11-35%, 3 - 36-70%, 4 - 71-100% of the root system infected.

** - Means followed by the same letter do not differ at P = 0.05 by Duncan's multiple-range test.

adhering soil and their roots were used to determine the effect of AA on rishitin accumulation and content of sterols.

Isolation of the free sterols fraction was performed by the method described by Metlitskii (1976). This method involves an extraction of PSs by a mixture of alcohol alkali and hexane, a selective deposition of sterols by digitonin, a disintegration by using dimethylsulfoxide of the molecular complex of digitonides with sterols and further identification of the sterols accompanied with an estimation of the total sterol content in the tomato tissues. Identification of PSs was done using gas-liquid chromatograph 'Chrom-5' filled with 'Laboratorni pristreje' (Czechoslovakia) and completed with a flame-ionization detector (the glass column filled with 3% solution of OV-17 based on chromatone NAWDMCS). Separation of PSs in the form of their acetates was done by GLS-mass spectrometry using a GLS mass spectrometer KRATOS MS-25-RFA (Great Britain). The glass columns were filled with a 1% solution of OV-1 and used in the appropriate analyses regimes.

A quantitative analysis was done to determine the concentration of sesquiterpenoid PAs in the tomato plants. This analysis was performed according to the procedure described by Avdyushko et al. (1988) which is based on GLS of the substances contained in tomato tissue extracts in purified alcohol and which does not require the production of volatile derivatives of sesquiterpenoids. Separation is done in a chromatograph 'Chrom-5', which is filled with 'Chromatron-N-Super' impregnated with a 3% solution of OV-225.

Squalan, and the preparations of rishitin, were used as the inner standard and as indicators, respectively. Twelve weeks after inoculation the experiment was terminated and fresh root and shoot weights were recorded. The root system were rated for galling using a 0-4 rating system, and, the numbers of eggs per gram of roots were determined for each plant.

The effect of AA on *M. incognita* infesting tomatoes was directly correlated with the AA concentrations (Table 1). All AA concentrations significantly depressed nematode galling. The numbers of eggs associated with the roots of plants grown from tomato seed treated in 0.1-1.0 ppm AA were significantly lower than those recovered from roots of plants grown from untreated seeds. However, the treatments with 0.1 ppm AA did not significantly suppress the number of galls and eggs produced. The development of nematodes was inhibited in all treatments with AA concentrations > 1.0 ppm although such AA concentrations were phytotoxic, as was obvious from retarded plant growth.

The concentrations of sterols in roots of plants grown from seed treated with AA were significantly lower than those in roots of plants grown from untreated seed. From a comparison of PA and sterol contents in experimental and control plants in our investigation it may be concluded that AA treatment of seeds modifies terpenoid biogenesis in tomato plants. Treatment of plants with 0.1 and 1.0 ppm concentrations of AA apparently initiated a plant response which resulted in increased rishitin production and which, in response to nematode invasion,

increased in concentration. Rishitin was not found in untreated control plants.

Seeds of a tomato cultivar susceptible to invasion and damage by *M. incognita* when treated with AA produced plants which apparently were able to develop a resistance mechanism to the nematodes. Furthermore, our investigation supports earlier suggestions that biogenic elicitors can function in two ways. Firstly, they can stimulate the production of rishitin to give increased concentrations in plants, and this substance is known to be nematotoxic (Zinovieva, 1993). Secondly, sterol deficiency can be induced in plants which much reduces the nutritional value of the plant to the nematode resulting in nematode death, as has been shown for free-living nematodes (Bolla, 1979).

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