Nutritional disorders in rice due to infestation by *Heterodera oryzicola* and *Meloidogyne graminicola*

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SUMMARY

Infestation of rice by *Heterodera oryzicola* and *Meloidogyne graminicola* caused reduction in nitrogen, phosphorus, iron, and reducing sugars in shoots and roots. Total sugars, protein, IAA, cytokinin and thiamine were reduced in the cyst nematode infested plants and phenols were reduced with root-knot nematode infested plants. Phenols decreased in shoots and increased in roots due to cyst nematode, while the rootknot nematode caused similar changes in potassium, manganese and magnesium. There was an increase in calcium, sodium, soluble amino-acids, ABA and starch in both shoots and roots of the cyst nematode infested plants and of total sugars, protein, DNA and RNA with the root-knot nematode infested plants. Infestation by both the nematodes caused nutritional disorders limiting the uptake of nitrogen and phosphorus and chlorosis of leaves.

RÉSUMÉ

Perturbations nutritionnelles chez le riz infesté par Heterodera oryzicola *et* Meloidogyne graminicola

L'infestation du riz par *Heterodera oryzicola* et *Meloidogyne graminicola* provoque une diminution de la teneur des racines et des parties aériennes en azote, phosphore, fer et sucres réducteurs. *H. oryzicola* diminue les taux de sucres totaux, de protéines, d'acide indol-acétique, de cytokinine et de thiamine, tandis que *M. graminicola* diminue le taux des phénols. Les phénols diminuent dans les racines et augmentent dans les parties aériennes dans le cas de *H. oryzicola,* tandis que *M. graminicola* provoque des modifications similaires pour le potassium, le manganèse et le magnésium. Le calcium, le sodium, les acides aminés solubles, l'ABA

1972). At 35 days after inoculation, the endoparasites in roots of fifteen plants were estimated (Rao *et al.*, 1971). Inoculated and uninoculated plants of both varieties were taken, the nematodes dissected out from the former and samples were adjusted to provide 10 g fresh weight of shoots or roots for analyses.

Samples of 1 g tissues of shoots or roots were cut into bits and boiled in 80 per cent ethanol. The supernatent was filtered and residues were repeatedly extracted to make 500 ml of filtrate (Yoshida *et al.*, 1976). This filtrate was used for estimation of total chlorophyll of foliage and a and b fractions (Arnon, 1959), total sugars (Hodge & Hofreiter, 1962), reducing sugars (Nelson, 1944), soluble amino-acids (Moore & Stein, 1948) phenols (Bray & Thrope, 1954) in mg/g. IAA and cytokinins (Chatterjee, Mondal & Sircar, 1976). ABA (Saavedra & Wain, 1974) and thiamine (Dasgupta & Cadwellader, 1970) were also estimated from the extracts and expressed as µg/g.

Sugar-free residues of the extracts were used to determine the starch (Hassid & Neufeld, 1964). Protein was precipitated in the extract (Racusen & Johnstone, 1961) and estimated in mg/g by modified Lowry's method (Bensadown & Winstein, 1976). Ethanol extracts were made lipid-free by addition of ethyl ether and centrifugation. RNA was precipitated from residues by alkaline hydrolysis (Howell, 1973) and the residues were further treated with 1 N perchloric acid for extraction of DNA fraction. Total RNA (Markham, 1955) and DNA (Burton, 1956) were expressed as µg/g.

Samples of shoots and roots were oven-dried in oven at 80° and powdered for determination of total nitrogen, phosphorus, calcium and sodium (Pattnaik, Misra & Bhadrachalam, 1966), potassium (Jackson, 1973) and iron (Sandell, 1950) and expressed as mg/g. Each chemical constituent in the nematised plant was presented as the percentage decrease or increase over the actual amount in shoots or root tissues of the healthy plant, given in parenthesis, for comparison.

Results and discussion

Cyst nematode

Nematode incidence

Infested plants showed typical symptoms in foliage and roots. The cysts and developing stages of the nematode were 28.3 and 58.5 respectively in root system, well above the threshold level of 34.5 endoparasites per plant (Rao, 1984).

Chlorophyll, sugars and starch

Leaf chlorophyll (4.87 mg/g) was reduced by 37.8 % in the nematode infected plant (Fig. 1). Consequently, total sugars in shoots (74.1 mg/g) and roots (34.9 mg/g) were reduced by 18.2 and 33 %. The amount of reducing sugars (54.7 mg/g in shoots and 24.9 mg/g in roots) was decreased by 13.5 and 34.5 % respectively. However, starch (5 mg/g in shoots and 1 mg/g in roots in healthy plants) increased by 77.3 and 68.3 %. Starch accumulation was similar to that obtained in root infestation by the lance nematode, *Hoplolaimus indicus* Sher, 1963 (Ramana, Prasad & Rao, 1976) root-lesion nematode, *Pratylenchus indicus* Das, 1959 (Prasad & Rao, 1978) and the root-knot nematode, *M. graminicola* (Mohanty & Rao, 1978) in rice. Reduction in chlorophyll inhibited photosynthesis (Livine, 1964).

Proteins, amino acids and nucleic acids

Total proteins (36.7 and 31.8 mg/g respectively in shoots and roots of healthy plants) were reduced by 36.7 and 31.8 % respectively in shoots and roots (Fig. 1). Soluble anino acids (22.9 and 12.7 mg/g in shoots and roots of healthy plants) had increased by 75.9 and 58.0 %. Enzymatic degradation of plant proteins and reduced photosynthesis caused accumulation of soluble amino acids and also reduction in the total number of tillers in the nematode infected plant. RNA (106.8 and 70 µg/g of shoot and roots) increased by 17.5 % in shoots and decreased by 25 % in the roots of nematode infested plants due to inhibition of protein synthesis and nitrogen metabolism of plants. DNA (14.8 and 15.6 µg/g in shoots and roots) showed a slight increase in shoots of infected plants by 1.3 % and in roots a decrease by 4.5 %. The non-interference by the nematode in DNA metabolism may be one reason for its inability to induce hypertrophy and gall development in the nematised plants.

Nutrients

Nitrogen content in shoots and roots of healthy plants was 74.9 and 69.8 mg/g respectively. Cyst nematode infestation caused a reduction by 20.3 and 40.1 % in shoots and roots (Fig. 1). Phosphorus (7.5 and 8.5 mg/g) was reduced by 38.3 and 26.6 % in shoots and roots of affected plants. Deficiency of nitrogen and phosphorus decreased the length of leaf (Ushakumari & Kuriyan, 1982), number of panicles and grains per panicle (Rao, 1984). Potash content (35.5 and 15.9 mg/g in shoots and roots of healthy plants) was reduced by 32.0 and 25.1 %. Reduction in potash decreased the thousand grain weight in nematised plants (Jayaprakash & Rao, 1981). The amount of iron in shoots and roots of healthy plants (19.0 and 14.7 mg per g) was reduced by 16.3 and 27.9 % in roots and roots of the infected plants. Deficiency of iron, an essential element for production of leaf chlorophyll, caused chlorosis. Calcium (19.0 and 10.6 mg/g in shoots and roots of healthy plants) had increased by 22.4 and 30.7 % due to nematode infestation. Similarly, sodium (4.3 and 1.9 mg/g) had increased by 27.1 and 127.3 % in shoots and roots of rice following infestation by the nematode. This indicated an increase in the absorption as a compensation for the decrease in potassium in order to maintain the cation balance as in the

case of potato *(Solanum tuberosum)* infected by the cyst nematode *Globodera rostochiensis* (Trudgill, Evans & Parrot, 1975).

Phenols

Total phenols in shoots and roots of healthy plants were 19.5 and 28.4 mg/g, and in the infested plants, the phenols were reduced in shoots by 35.7 % and increased in roots by 31.5 % (Fig. 1). Basipetal translocation of free phenols at sites of nematode attack and establishment in roots, and the interference in the phenol metabolism by the nematode, contributed to the reduction of phenols in the shoots.

Growth regulators and hormones

IAA (13.0 and 10.2 microgrammes/g) in shoots and roots of healthy plants) was reduced by 39.2 and 32.4 % (Fig. 1). As gall formation was not essential for the development of the endoparasite, the levels of indole compounds were low, which however caused a general reduction in height of plants infected by the nematode (Kuriyan, 1985). Cytokinins (14.2 and 12.1 µm/g of shoot and root of healthy plants) were also reduced by 63.6 and 43.0 % indicating a disruption in their synthesis and translocation, causing loss of chlorophyll. The ABA levels (0.9 and 0.8 µm/g of shoots and roots in healthy plants) had significantly increased by 3.18 and 2.76 times in shoots and roots of infected plants and this increase caused stunting, destruction of chlorophyll, accumulation of starch and inhibition of plant growth hormones such as IAA and cytokinins (Addicott & Lyon, 1969).

Vitamins

Thiamine (Vitamin B 1) content in shoots and roots of healthy plant was 14.5 and 11.0 µm/g. Thiamine was reduced by 40.8 and 47.3 % in shoots and roots of nematode affected plants (Fig. 1). Disturbance in the normal synthesis of thiamine in roots and its translo-

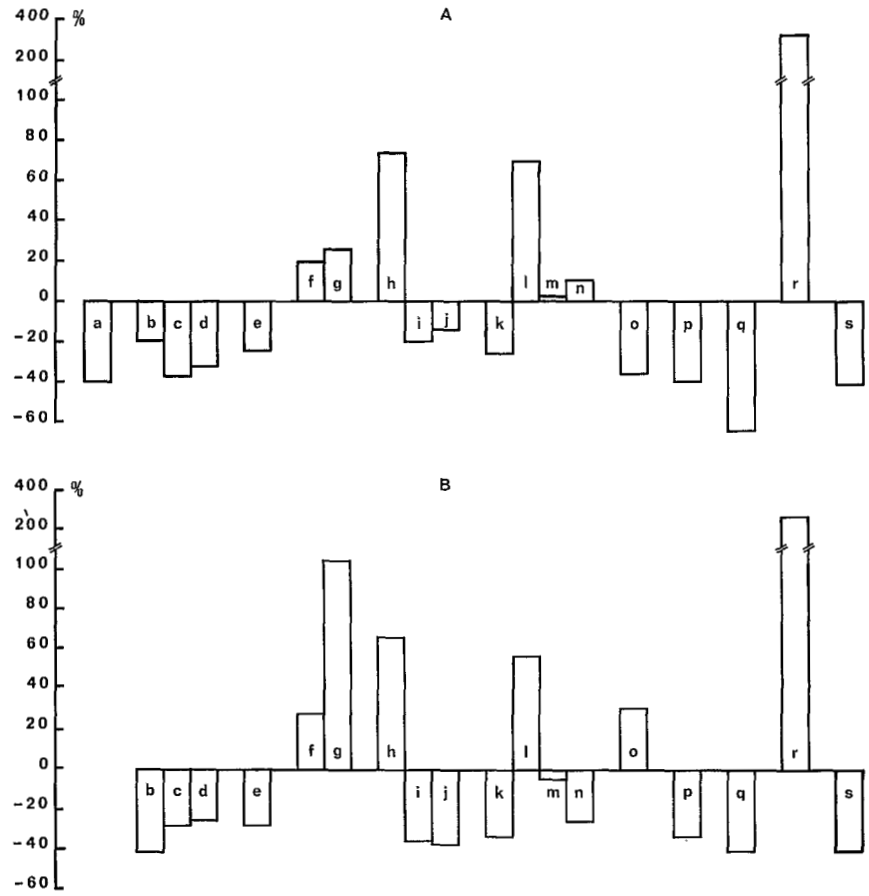

Fig. 1. Changes in the chemical constituents of rice plants, 35 days after inoculation with *Heterodera oryzicola;* A : shoot; B : root. (a = chlorophyll; b = nitrogen; c = phophorus; d = potassium; e = iron; f = calcium; g = sodium; h = starch; i = total sugars; j = reducing sugars; k = total protein; l = soluble amino-acids; m = DNA; n = RNA; o = phenols; p = indol-acetic acid; q = cytokinins; r = IBA; s = thiamine).

cation to roots was found to cause reduction in root growth.

Increase in calcium, sodium, starch and ABA and decrease in nitrogen, phosphorus, potash, iron, total and reducing sugars, protein, IAA, cytokinin and thiamine in both shoots and roots characterised the changes in the nutritional status of rice plants infested by the cyst nematode. Drought conditions in maize (*Zea mays* L.) and viral infection in rice increased ABA synthesis (Saavedra & Wain, 1974). Decrease in phenols of leaves and leaf-sheaths may be one reason for the increase in

and roots. The number of endoparasites and egg masses were 64.5 and 31.5 per plant, higher than the threshold level of 15 egg masses per plant (Rao, 1984).

Chlorophyll and sugars

Total chlorophyll in leaves of healthy plants was 3.7 mg and of a and b fractions were 2.1 and 1.6 mg/g. Chlorophyll was reduced, owing to root-knot nematode incidence, by 30.2 % of total and 39.5 and 32 % of the a and b fractions. Total sugars (5.2 and 2.8 mg/g in shoots and roots of healthy plants) increased by 36.6 and

plants) was reduced in shoots by 29.5 % and increased in roots by 24.5 %. Similarly, manganese (0.6 mg/g in shoots and 0.1 mg/g in roots of healthy plants) was reduced in shoots by 48.0 % and increased in roots by 3.63 times in the nematised plants perhaps due to low absorption of iron (Pande, 1984). Deficiency of calcium, magnesium and phosphorus in early growth stages of the plants interrupted the normal physiological functioning of roots.

Phenols

The phenol content of shoots and roots of healthy

EPSTEIN, E. I. (1974). Biochemical changes in terminal root galls caused by an ectoparasite *Longidorus africanus*. Nucleic acids. *J. Nematol.*, 6 : 48-52.

HASSID, W. Z. & Newfeld, E. F. (1964). Quantitative determination of starch in plant tissues. In : Whistler, R. L., Smith, R. J., BeMiller, J. N. & Wolfrom, M. L. (Eds). *Methods of carbohydrate chemistry* IV., New-York, Academic Press : 33-36.

HOWELL, H. S. (1973). The isolation and analysis of DNA from eukaryotic cells. In : Chrespeels, M. J. (Ed). *Molecular techniques and approaches in developmental biology.*

PATNAIK, S., MISRA, C. B. & BHADRACHALAM, A. (1966). The analysis of mineral nutrients in plants. *Lab. Manual. CRRI, Cuttack*, 30 p. [Mimeogr.].

PRASAD, J. S. & RAO, Y. S. (1978). Metabolic changes in rice plant (*Oryza sativa* L.) induced by the root-lesion nematode (*Pratylenchus indicus* Das). *Proc. Symp. Host Parasite Relationships, Univ., Madras* : 19 [Mimeogr.].

PRASAD, J. S. & RAO, Y. S. (1981). Histopathology of rice roots infected with *Pratylenchus indicus* Das. *Riv. Parassit.*, 42 : 475-481.

RAMANA, K. V., PRASAD, J. S. & RAO, Y. S. (1976). Biochemical cchanges in roots of rice infected by the lance nematode, *Hoplolaimus indicus* Sher. *Indian J. exp. Biol.*, 14 : 216-217.

RAMANA, K. V., PRASAD, J. S. & RAO, Y. S. (1976). Biochemical changes in roots of rice infected by the lance nematode, *Hoplolaimus indicus* Sher. *Indian J. exp. Biol.*, 14 : 216-217.

RAO, Y. S. (1984). *Nematode problems in submerged soils and their management.* Haryana Agric. Univ., Hissar, 12 p. [Mimeogr.].

RAO, Y. S. & BISWAS, H. (1973). Evaluation of yield losses in rice due to the root-knot nematode. *Indian J. Nematol.*, 2 : 72-76.

RAO, Y. S. & ISRAEL, P. (1972). Influence of inoculum density on the final population of the rice root-knot nematode. *Indian J. Nematol.*, 2 : 72-76.

RAO, Y. S., ISRAEL, P., RAO, V. N., RAO, Y. R. V. J. & BISWAS, H. (1971). Studies on nematodes of rice and rice soils. III. Extraction methods and sampling. *Oryza*, 8 : 65-74.

SAAVEDRA, A. L. & WAIN, R. L. (1974). Abscisic acid levels in relation to drought tolerance in varieties of *Zea Mays. Nature*, 251 : 716-717.

SANDELL, E. B. (1950). Iron. In : *Colorimetric determination of traces of metals.* New-York, Interscience Publishers : 95-103.

SRIDHAR, R. & OU, S. H. (1974). Biochemical changes associated with the development of resistant and susceptible types of rice blast lesions. *Phytopathology*, 2 : 79.

TRUDGILL, D. L., EVANS, K. & PARROTT, D. M. (1975). Effects of potato cyst nematode on potato plants. I. *Nematologica*, 21 : 169-182.

USHAKUMARI I. A. & KURIYAN, A. J. (1982). Cyst nematode *Heterodera oryzicola* on rice in Kerala. I. Estimation of losses. *Indian J. Nematol.*, 11 : 106 [abstr.].

YOSHIDA, S., FORNO, D. A., COOK, J. H. & GOMEZ, K. A. (1976). *Laboratory Manual for Physiological Studies on Rice.* IRRI, Manila, Philippines, 82 p.

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